STUDIES UPON CALCAREOUS DEGENERATION.[1]

I. THE PROCESS OF PATHOLOGICAL CALCIFICATION.

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INTRODUCTION.

It is a well known fact and a not uncommon occurrence that the interest in certain subjects or lines of thought fluctuates widely; we are ever too ready, when we think we have obtained an explanation of a certain phenomenon, to take much for granted and allow our enthusiasm to lapse. Such has been the case in the study of pathological calcification, which has gone through several periods of new theories and rest.

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Notwithstanding these theories, in reviewing the literature concerning calcareous degeneration or calcification, one is struck with the dearth of actual facts that are set forth upon which those theories are based. In truth the labor that has been spent in the actual study of the *process* has not been extensive and it is confined to a few papers in the last decade. There have been many who have reported the occurrence of calcareous degeneration of diverse tissues and have even gone minutely into the chemical nature of the organic salts present, but here the study has ended. Regarding the existence of pathological calcification we have abundant data, as to the process of calcification we know little.

Indeed, calcification is one of the commonest pathological conditions noted by the older writers, especially that occurring in the arteries and in fibromata of the uterus. Not infrequently the change in the arteries is spoken of as appearing "partly fatty and partly earthy." No doubt reference was made to the yellow atheromatous areas overlying calcareous plaques in the arterial wall. Not until Virchow in 1858 asserted that inorganic salts were deposited in supersaturated solutions of the same and that the deposit of calcium was of this nature, was there any discussion on the subject. Virchow held that calcareous deposits were chiefly found in old age and in diseases causing wasting of the bony framework, and that in this state calcium was dissolved out of the osseous system and on account of the too concentrated solution of calcium salts in the blood these were laid down elsewhere as metastatic deposits. In this way he explained the common occurrence in old people of calcareous plaques in the arteries.

Some years later von Recklinghausen reported the studies he had made upon the degenerations. He pointed out that calcium deposits result partly in tissues which are well supplied with tissue fluid, but also in tissues poorly supplied. When blood or tissue fluids are saturated with calcium salts, due to repeated breaking down of bony structures, he noted a secondary incrustation of the lung and kidney tissue. But there were other cases in which, as in fibroids and phleboliths, calcification proceeded under conditions of normal calcium content of the

blood. Von Recklinghausen looked on the processes of ossification and petrification as similar chemically, though differing biologically, in that the former took place in active and living cells, the latter in degenerating or dead tissues. He held that the factors governing calcification were: (i) the separation of sparingly soluble lime salts, (ii) the existence of these substances in excess in the fluids, and (iii) the presence of a foreign body which acts as a nucleus.

These factors would be of weight in considering fluids outside the body but not within. The lime salts in the tissue fluids are not found in excess during calcification and the common salts of lime found in the tissue fluids are very soluble. The deposit of calcareous plaques in the serous membranes and in the arterial walls he considered under ossification and as distinct from calcification.

Lancereaux's views were very similar to those of von Recklinghausen, though he attributed the supersaturation of lime salts in the blood to a hindrance of the excretion of these salts by the urine, and found them accumulated in the pyramids of the kidney and the mucosa of the stomach and intestines.

Thus it was that up to the time of Litten's ingenious experiments (1881), the theory of the excess of lime salts in the blood was accepted as explaining calcareous degeneration. No note was taken of the predilection of certain tissues for this deposit of lime, except that dead or dying tissues were regarded as a nucleus about which the carbonate and phosphate of calcium would crystallize. With Litten's studies—which will be discussed more fully later—a new theory was raised which superseded that inaugurated by Virchow, and up to the present time his has been the explanation of calcification accepted in the textbooks. After the appearance of Litten's work many followed in his footsteps, the majority supporting his views, although some have disagreed with him. The prevalent view, based on that of Litten, is briefly (dealing particularly with the cases of calcareous degeneration in the kidneys) that one or more irritants act on certain tubules in the kidney through which these irritants are excreted, and during their passage through the kidney cells,

so injure the tubular epithelium that a degeneration of the cell protoplasm occurs, and that this degenerated cell protoplasm has an affinity for, and fixes as an insoluble compound, the calcium salts brought to it by the lymph and blood. These, it is maintained, are the essentials for the deposit of calcium salts, and it is pointed out especially that a coagulation necrosis of the renal epithelium is a necessary stage in the process.

This view has been generally accepted; and yet it is noteworthy that no one has proved that outside the body there is to be obtained the formation, even temporarily, of compounds of lime and any form of proteid. The present investigation was undertaken, in the first place, to determine with what organic substance present in degenerating tissues it could be shown that lime salts entered into combination. It was in repeating Litten's and von Kossa's experiments and in studying also the changes occurring within celloidin capsules containing various substances, and introduced into the peritoneal cavity, that indications were gained leading to the results about to be detailed. It was first undertaken to produce, experimentally, calcification of the kidney and other tissues and to study the successive changes to be noted in these. The rabbit was most frequently used in these experiments, partly on account of the normally high calcium content of the blood, and partly because these animals stand various operations very well. But in addition many calcified tissues from the human body were studied, the process in connection with arterio-sclerosis especially, and, as will be detailed, not a few experiments of other nature were made during the course of the investigation.

In studying the calcareous degenerations, the tissues were subjected to a series of tests as to the staining qualities and the chemical nature of the material. From all the experimental work on animals and the analyses of human degenerating tissues, one conclusion was always arrived at, namely, that calcareous degeneration is preceded or accompanied by deposits of a soapy material. This soap exists in chronically inflamed tissues in advance of the deposit of lime, or, if the lime is already to be found in the tissues, the soap appears in the peripheral zone

of the calcareous infiltration. Moreover, it would seem that with the first deposit of soap, calcium salts are immediately attracted to it from the body fluids. Such progressive calcareous deposits are marked by the appearance in the periphery of groups of granules, some of which are found to react for both calcium and soap, others for soap only,—that is, some of the soap deposit has been converted into a compound containing calcium; in other words, calcium exists in the recently calcified areas as calcium soap.

That this calcium soap does not continue to exist as such was noted in old calcified fibroids and other tumors. In these it was seen that, except in the advancing border of calcification, the dense calcareous deposits are composed chiefly of calcium carbonate and calcium phosphate with no soap in its substance. It would seem that the fatty acid moiety of the calcium soap had here been replaced by the stronger phosphoric and carbonic acids to form the more stable compounds.

This is, in brief, the trend of the paper.

Before proceeding with the description of the work I wish to extend to Professor Adami my best thanks for the supervision of the entire work and for his unfailing help and the many suggestions which have been of great aid to me during the course of this and other researches.

STAINING METHODS.

Calcium and Calcium Compounds.—As a routine all specimens were stained with hematoxylin and eosin. These stained specimens gave the general character of the section, and, moreover, the presence of calcium salts, when in fair amounts, was indicated by the appearance of fine blue-black dust-like granules, which stained darker than other portions of the tissue and were easily recognized. The hematoxylin will, however, only stain calcified areas when calcium salts are present in very fair quantities; slight amounts are unrecognizable by this stain.

To demonstrate calcium salts in tissues we have two other methods, one of which was of the highest service in my hands.

If tissues containing lime salts be treated for five minutes with a solution of pyrogallic acid (1 to 40 in water), to which one per cent. of sodium hydrate has been added, the lime salts will be stained a dark seal-brown while the remaining tissue appears a light-yellow color. Except in tissues where large quantities of lime salts are present, the stain is unsatisfactory, just as in many cases which show the presence of calcium salts with the following method the pyrogallic acid treatment gave no results. The most satisfactory method we have used is that recommended by von Kossa, namely, the treatment of sections with a 5 per cent. aqueous solution of silver nitrate. This solution picks out the most minute quantities of calcium by depositing an intense black precipitate in its place. The sections were immersed in the silver solution for from 3 to 12 hours, then washed in distilled water, and mounted in the usual way in Canada balsam. As the silver treatment of sections consists of an impregnation and precipitate in the tissues, and not a stain, we obtain a much exaggerated picture of the actual quantity of lime salts present. However, this is of all the more service when minute quantities of earthy salts exist, and the method is not proposed as affording a quantitative measure.

As was pointed out by von Kossa, the blackening produced by the treatment of sections with silver nitrate is due to the production of silver phosphate which blackens in the presence of light; hence it would seem that a precipitation of silver is only obtained in the presence of a phosphate. The calcium deposit in the tissues exists, however, both as a carbonate and a phosphate, principally the latter, and this experiment would only lead us to detect the insoluble calcium phosphate. However, when sections containing calcium carbonate are treated for many hours with silver nitrate a certain portion of the two salts interact so that the calcium-carbonate granules become coated with a deposit of silver carbonate which in sunlight gives off carbon dioxide leaving the black silver oxide, or we can hasten the stage of blackening when some silver carbonate has formed by first thoroughly washing the specimen in distilled water and then treating it with a dilute soluble sulphide.

There is, however, I would point out, a means whereby the calcium existing in any favorable combination in the tissues can be demonstrated by the silver-nitrate method; and this consists in first converting calcium salts into the chromate by means of potassium chromate and then replacing the calcium by the silver, thus obtaining the silver chromate which blackens on exposure to sunlight. Under ordinary conditions the calcium chromate is soluble in water, but when tissues containing calcium are acted on in the cold we find that the calcium chromate does not leave them, and on further treating them with silver nitrate the red silver chromate is seen to become deposited in their sites. The use of silver nitrate in these impregnation tests is we have found facilitated by acidulating it with acetic acid. This same method demonstrates calcium when combined with fatty acids to form soaps.

I would point out that these micro-chemical tests for calcium do not demonstrate the actual calcium salts, but by replacement we demonstrate their presence and location with blackened silver salts.

Virchow and Halva have both analyzed calcareous nodules by chemically demonstrating the presence of both phosphate and carbonate of lime. However, they point out that the phosphate is much in excess; while Chiari found only phosphatic salts in the calcified nodules examined by him.

The method of staining lime salts with silver nitrate gave beautiful pictures in all of the tissues examined. Especially was this true of the sections of experimental calcification of the renal tubules (vide Fig. 2, Plate xxx.). It is true that the metals, mercury, copper, and lead, will give a black precipitate in tissues with silver nitrate, but they can be excluded in these cases when other evidences are given of the identity of the calcium.

However, in the experimental production of calcium deposits in the kidney we have to deal with another factor not often met with in the slowly forming calcareous nodules. In the former we are inducing a sudden change by means of chemical or mechanical reagents, and this has an effect on the general metabolism. Especially is this so in the inoculation of animals with such

poisons as corrosive sublimate, lead acetate, or copper sulphate. Here besides acting directly on the kidney tissue we produce an hæmolysis of the red blood cells and thus occasionally, depending on the strength of the inoculation, we find an iron deposit in the same convoluted tubules, which are the seat of calcareous degeneration. Similar though less amounts of iron can be demonstrated in the liver; but that the presence of iron in the kidney is not the cause of the silver deposit, is to be ascertained from the fact that no calcium reaction results in the liver. The iron in both instances is detected by the Prussian-blue test. Attention has already been called by Gierke to the occasional presence of iron in specimens of pathological calcification.

In the routine examination of aortas we were struck by the frequency of stainable calcium salts found in either the media or intima where no lesion was suspected from the macroscopic or ordinary microscopic examination.

Fats and Fatty Compounds.—For the detection of fats, fatty acids, and soaps, I found that Sudan III gave the most satisfactory results. I have also used Scharlach R., which is a very closely allied stain to Sudan III, but found a difficulty in the employment of it as the stain precipitates very readily in its crystalline form into the tissues. Scharlach R. is the more intense but cannot be used in saturated solutions. Osmic acid impregnation of tissues to demonstrate fats is of more limited use, as we know that only the oleic acid and olein are blackened by it, while stearic and palmitic acids with their respective fats are left untouched. Hence in this work, outside of making comparisons of the quantities of olein or oleic acid and the total quantities of fats and fatty acids, sections were stained with Sudan III; osmic acid was used only very occasionally. The nature of Sudan III and Scharlach R. is such that they are insoluble in water but are soluble in alcohol, chloroform, ether, fats, fatty acids, and soaps. A saturated solution of the stain is readily obtained in 70 per cent. alcohol, and as the fats, fatty acids, and soaps are more ready solvents of it than is 70 per cent. alcohol, fat in sections treated with a saturated solution of Sudan III will pick out the stain while the rest of the tissue remains

untouched. Our sections were usually stained 12–24 hours in the cold. (Vide Fig. 1, Plate xxx.)

Hence in Sudan III we have a stain which will differentiate fats, fatty acids, and soaps from other products in tissues. That soaps are ready solvents for Sudan III is readily demonstrated by making a concentrated watery solution of a soap and then adding the powdered Sudan III. The resultant mixture is a clear, deep red solution which can be filtered and further diluted. Such a stained though highly dilute solution, when evaporated on a slide gives a pinkish-yellow color, which is distinct from the golden-red staining of neutral fat. Fat particles even when emulsified to fine droplets retain this intense golden red appearance. The difference between the Sudan III fat stain and Sudan III soap stain is quite readily recognizable so that the two can be detected in tissues. It is more difficult to distinguish the neutral fats from the fatty acids in stained smears under the microscope, though olein appears distributed over the slide as stained oil globules which are easily picked out. These latter can be further differentiated by treatment with osmic acid.

It is to be noted that fatty acids as such are infrequent in the tissues, except in the case of dead tissues, such as gangrenous areas and pus, or as in the process of splitting up of fats in what are termed fat necroses. It is obvious that living cells do not pick up fatty acids unless, according to Fischler, it be leucocytes, in which case we can at times demonstrate fatty particles in the cytoplasm and show the vacuoles about the particles to be of acid reaction.

Fischler is most emphatic in his assertion that Sudan III is soluble in soap solutions, and the color produced on solution distinguishes this substance from the fat when stained in the same way, or, in other words, that fat and soap granules are to be differentiated in stained tissues. My results wholly confirm these statements. He states, further, that soap, when stained with Sudan III and then treated with 70 per cent. alcohol, immediately loses its color, the dye being taken up by the alcohol. This I failed to confirm wholly.

The statement is true of soap preparations in the test-tube, but, as we shall see later, soaps present in the tissues do not dissolve so readily in weak alcohols; hence they retain the stain after this treatment. As will be pointed out, these intracellular soaps do not exist as a free product, but form combinations with albumin with coincident alterations in reaction.

Calcium soaps are also stained by Sudan III, giving color reactions similar to those of potassium and sodium soaps. Calcium soaps, appearing in the tissues as a new deposit in which there is no excess of free calcium salts, are not distinguishable from other soaps. But when a portion of the calcium is freed and deposited either as carbonate or phosphate, we find, among the Sudan-III-stained soap granules, other, crystalline, bodies, highly refractile and not absorbing the stain. The latter granules, it is found, will precipitate with silver-nitrate solutions. In tissues therefore it is possible to treat the sections first with Sudan III, and later with silver nitrate, demonstrating the presence of soap granules and also showing amongst these free calcium salts not associated with fats.

Some might be sceptical regarding the ability to distinguish the finest fat particles from the granules of soap stained in sections. Such scepticism we can override by another method. If the material be cut by the freezing microtome, the sections quickly dehydrated in 70 per cent. alcohol, then washed with petroleum ether, the tissue may be rid of all the microscopic fat. If now soaps be present, the soap reaction with Sudan III will still persist. I do not wish to say that the ultimate fat is removed from the tissue, but all the stainable fat is taken out, and the petroleum ether has been without action on the soaps. Rosenfeld and Dormeyer have pointed out that tissues which do not show fat microscopically, when washed with ether or petroleum ether, still contain a considerable quantity of fat present within the cells; but the combined fats of this order give no reaction with Sudan III; they may be neglected in this regard. Here it may be noted that even after decalcified tissues have been prepared in the usual way for cutting in paraffin, by dehydrating in absolute alcohol and clearing with xylol, sections will still stain

faintly with Sudan III in the regions of active calcification. I cannot but believe that in such cases we have to deal with soaps rendered less soluble by their combination with albumins (vide p. 659).

CHEMICAL METHODS.

The Determination of Soaps.—In our first experiments the method of Hoppe-Seyler was employed. The tissue under examination was finely ground up and extracted with boiling absolute alcohol. From the filtrate, evaporated to dryness, fats were later removed by ether or petroleum ether, and the residue was demonstrated to contain sodium and potassium soaps, along with small quantities of calcium soap. The residue on treatment with mineral acids yielded stainable fatty acids, all dissolving in water; the sodium and potassium soaps were precipitated with calcium chloride. As calcium soaps are so slightly soluble in alcohol, only small quantities of them were thus obtained and we had to rely on the flame test to demonstrate the calcium present.

The use by this method of hot alcohol for the extraction leaves it possible, perhaps, that a small amount of neutral fat may be converted into soap by means of the alkalies present in the tissues. As I was, however, dealing with calcified tissues, such as fibromata of the uterus, from which all the surrounding superfluous tissue had been removed and which were then washed with distilled water, I can hardly believe that sufficient sodium or potassium remained in the tissue to give rise to the formation of soluble soaps.

To obviate this possibility of error, the method was given up and the fats were extracted without the use of heat. After the material had been finely chopped it was dried over sulphuric acid and then extracted with petroleum ether in many relays, the powdering of the tissue being undertaken between each treatment with fresh petroleum ether. Dormeyer is very emphatic in his assertions that this method of treating tissues with ether, even when carried on for a long time, does not remove the last trace of neutral fats contained within the cell membrane. He

prefers to digest with pepsin and 0.5 per cent. hydrochloric-acid solution, and thus break up all the cell bodies, whereby he claims to obtain 8.5 per cent. more of fat.

As the analyses here undertaken were not of a quantitative but only of a qualitative nature, these minute estimations are of no bearing. However, the gross fats being once removed, the powdered residue was treated with hot acid alcohol to dissolve out the soaps (which had been unaffected by the petroleum ether), and a filtrate obtained. From the filtrate the soaps were precipitated with acid calcium-phosphate solution, filtered off, and washed with ether. In tissues actively undergoing calcification, this residue was obtained in fair quantity—in fact, in greater quantity than in densely calcified tumors in which the amount of the calcium salt was altogether out of proportion to the fatty acid. The alcoholic extract of the residue, after treatment with ether and petroleum ether, yielded small quantities of calcium soap, which we must remember is only very slightly soluble in absolute alcohol.

THE PROCESS OF DEPOSIT OF CALCIUM SALTS IN BONE.

In Normal Bone.—In the above description of the methods employed I have had to refer to the results obtained, and, if only in a general way, I have indicated that, in tissues undergoing calcification, fats, soaps, and insoluble calcium salts are found associated in varying proportions, according to the stage of the process. We will now turn to a more detailed study of the process, and first consider whether under normal conditions—when calcium salts are laid down in bone—there is to be made out this same association; whether, in short, ossification and pathological calcification are processes of the same order.

The phalangeal bone of an infant six days old was examined by sectioning on the freezing microtome and staining with Sudan III. The head of the bone was cartilaginous with a centre of ossification within it. The sections were allowed to stain for eighteen hours, but at the end of this time did not take the Sudan III stain in any part. Both the cancellous and the compact bone were devoid of anything resembling fats, fatty acids, or

soaps. The femur of a fœtal guinea-pig was next sectioned and treated in the same way and with like results; that is, we found that in normal growing bone there appears to be no stage in which fatty substances play any part in the deposition of the lime salts, and we are led to believe that this laying down of calcium salts in the bone is a secretory process on the part of the osteoblasts. Again, in sections of decalcified skull bone we found the matrix entirely devoid of microscopical fatty substance. The bone corpuscles, or lacunæ, remained unstained with Sudan III; nevertheless there was some neutral fat lining the Haversian canals and surrounding the contained blood-vessels. Thus we conclude that *the process of calcification in normal growing bone is a distinct process in which the specific cells of the part play the chief rôle in laying down the calcium salts*. There was nothing in the stained tissue which would lead us to believe that there is any connection between fatty substances and the advancing bone-formation at the epiphyseal line.

Pathological Bone Formation.—What is true with reference to normal bone was found to obtain also in new bone developed under pathological conditions. For this purpose we examined a small bony nodule developing in the tissues to the inner side of the lower end of the humerus, secondary to trauma and due apparently, as pointed out by Dr. Archibald, to a local separation of portion of the periosteum. In the second place, we examined a portion of a large nodular, pure osteoma of the left frontal bone, obtained from a man twenty years old, which had grown into the cranial cavity and had produced great atrophy of the frontal lobe. [Royal Victoria Hospital—P. M. Reports, No. $^{15}/_{05}$.] In neither of these specimens did Sudan III give any trace of the presence of fat or soap in connection with the more recently growing portions.

THE PROCESS OF DEPOSIT OF CALCAREOUS SALTS UNDER PATHOLOGICAL CONDITIONS.

In the Arterial Wall.—We now pass on to tissues which, under pathological conditions, undergo *calcareous degeneration*. Of

these first and foremost are the arteries. All specimens examined by us, coming from persons over twenty-five years of age, showed more or less change in the aortic wall. The change varies from the faint white streaks which are seen through the intima, to the condition in which calcareous plaques are present, with and without ulceration.

The most remarkable point that strikes one in staining a series of aortas by the methods described, is the frequent presence of recognizable amounts of calcium salts in the media of adults. (Vide Figs. 3, 4 and 5.) In the routine examination of eleven specimens of the aorta, from persons varying in age from twenty-two to forty years, which on macroscopical examination showed no lesion other than fatty plaques in the intima, it was found that calcium salts were present in abundance at one time in the media, at others in the intima. It was not infrequently seen that the media was affected by the calcium increase while the intima remained unchanged or was affected by a slight extension only of the process from the media. We hope to call fuller attention to this change in the media in a subsequent article. So far it would seem to have escaped notice.

The white plaques in the intima have long been noted by pathologists and have passed under a host of names. Gross, as early as 1845, spoke of the condition as "atheromatous" and "steatomatous," where he says the microscope "brings into view a multitude of crystalline plates and fatty granules with albuminous and oily particles." Coplin has collected cases in which the change in the arterial wall was noted as early as the second year of life, but it is not our intention to deal here with the frequency of this affection. What is to be noted is that all vessels with arteriosclerosis have stainable calcium salts in either intima or media to a greater or less extent.

In studying normal aortas, taken from young adults, we found that neither intima nor media was affected by Sudan III, while only a few fat cells were seen in the adventitia. That is, in a normal and healthy state the intima and media of the aorta show no "microscopic" fat, and, further, when such aortas are stained with silver-nitrate solution there is no staining or deposit of silver

in the tissues. Obviously the small amount of calcium salts and fat normally present in the tissues is not detected by our staining methods.

Sections were made from the iliac artery of an adult aged thirty years, which was apparently healthy on gross inspection, with the exception that here and there white areas of the size of a pin-head and not raised above the surface were present in the wall. The specimen was one which had been picked from a number as a sample of an apparently normal large vessel of adult life. When stained with hæmatoxylin, it was seen that the elastic layer did not follow the lumen of the vessel at an equal distance around the artery, but at the sites of the macroscopic fatty areas the intima was thickened towards and bulged into the media, and here the elastica was distorted and at times broken. Away from these isolated changes in the intima, the artery was found to be perfectly healthy. When stained with Sudan III, it was found that these thickenings showed the presence of numerous fatty granules, many of which stained an intense reddish-yellow. This fatty material lay in aggregated clusters of granular particles of larger and smaller size, situated chiefly at the border-zone of the intima, next to the media. While these fatty particles were seen in the nodular portion of the intima, the rest of the arterial wall was free.

In the healthy portion of the arterial wall the elastic lamina did not stain with Sudan III, and followed the lumen of the vessel at an equal distance and in a single strand. As we approached the intimal lesion, the elastic layer split longitudinally into a number of strands, which now, instead of retaining a regular wavy contour, became of unequal undulations and spread apparently into the media. The individual strands of the elastic lamina were swollen and fragmented, showing in places "a snapping" of the fibres. In this area of change in the elastica, the fibres were also seen to take the Sudan III stain, which became more intense and more granular as we approached the thickening of the intima. Immediately below the fatty deposits in the thickened intima, the elastic fibres were found much split up and composed of short, curly, Sudan-III-staining, strands.

When we come to examine closely the particles stained by Sudan III, we find them of different characters. First, we note the larger droplets or granules which are not perfectly round and which are isolated, or else we see several of these fusing into larger masses. The color of these is unmistakably that of neutral fats. Besides these we find many more smaller particles which are distinctly semi-crystalline and of a much lighter color, approaching almost to pink. These pink-staining particles are found in greatest abundance along the border-zone between the intima and media, and often occupying the elastic lamina itself.

It was found that when consecutive sections were stained alternately with Sudan III and silver nitrate, the silver nitrate was precipitated in areas identical with those picked out by Sudan III, though the relative quantities were unequal, there being proportionately more fat present. That is, the calcium deposit picked out by the silver nitrate occupies the nodular portion of the intima and is not seen in the rest of either intima or media. Further, the portion of the thickened intima picked out is that occupied by the pink-staining granules. In the less affected parts the calcium appears as fine dust-like material staining an intense black. Judged by the outline, seen as a silhouette against the lighter background, the individual granules here are crystalline. In places the elastic lamina was picked out as a wavy line composed of aggregated granules of calcium.

In this specimen we have the early change in the aorta, a condition which we would not yet speak of as arteriosclerosis. The white pin-head patches on the intima are seen to represent areas with fatty substance of two kinds: one an intensely yellowish-red staining material, which is fat; and lighter particles, which from their reaction with Sudan III can only be soaps; and, further, the thickenings in the intima are shown to contain an increase in the calcium content, and this increase to occur in conjunction with the light, soapy staining material. In other words, the calcium increases side by side with the soap; and the arrangement described suggests strongly that we are dealing with a deposit of calcium soap.

In another instance there was a generalized thickening of the

arteries, though only at the bifurcation of the aorta was any macroscopical calcareous degeneration to be seen. The aorta showed numerous white plaques raised above the surface, and one of these from the abdominal aorta was sectioned. Stained with Sudan III both media and intima showed numerous aggregations of stained particles; the staining in the intima was confined chiefly to the atheromatous thickening in which considerable fat-staining masses occurred, while outside the nodule the granules were collected mainly round the elastic lamina. As in the previous case the granules could be divided into two classes: the larger masses which fused with other contiguous particles, readily recognized as fat deposits; and the smaller, lighter-staining granules which appeared semi-crystalline in shape. In the intima the larger fat masses were in excess of the finer granulations, while in the media the reverse condition existed; that is, the light-staining granules were much in excess of the fat, in fact only a little fat was to be seen on the boundary between the media and intima. In the rest of the media there were clusters of fine sand-like particles lying between the elastic layers and forming rows, more or less wedge-shaped, which occupied the position of the muscular fibres. In the media the stained granules were intermingled with numerous unstained refractile bodies, which disappeared from the specimens when treated with acid solutions. On treating the sections with silver nitrate it was found that the blackened deposit was present in the media in the regions where the Sudan III demonstrated the light-staining soapy particles. Similar to the Sudan III granules, the silver-nitrate deposit was aggregated into clusters lying parallel to and between the elastic fibres. The silver-stained particles in these clusters were much in excess of the refractile bodies seen in the Sudan III specimens; they tallied in number with those seen in unstained sections. The distribution of the silver-stained calcium salts was fairly general through the media, the greatest amount being towards the internal surface. (Vide Figs. 3 and 4.)

In the intima, however, there was less calcium to be seen, that present being confined to the atheromatous areas where the deposit seemed to be invading from the media. Beyond the

atheromatous plaques, there was no lime salt to be seen in the intima excepting a fine, dust-like infiltration along the boundary zone. The whole condition in the arterial wall we look on as consisting of two distinct processes: the change in the media of a chronic nature and of longer standing than the intimal change, the degenerative process with fatty change having been progressive; and the atheromatous condition in the intima of more recent date, owing to which time had not been given for such extensive deposits of calcium salt.

We now undertook to remove all the neutral fats and fatty acids from the sections of the aortas above described by treating them with ether and petroleum ether. They were first washed in 70 per cent. alcohol before subjecting them to the ether. After this treatment the only material remaining which could stain with the Sudan III would be a soap. On examining the sections stained with Sudan III, it was found that all the bright-red staining neutral material had been removed and all that remained to take the stain were small crystals of a light salmon-red color. These crystals lie in clusters of 50 or more, the long axis of the clusters being parallel to the direction of the fibres. The clusters are to be found mostly in the intima at the atheromatous area and in this tissue lie some little way inwards from the endothelium. In the deeper parts of the intima the clusters become more broken up, and after staining with silver nitrate are seen to be mingled with calcium deposits, which are absent in the more superficial parts of the intima. Hence the Sudan-III-staining particles which remained near the boundary zone between the media and intima after treatment with petroleum ether are combined with calcium salts, while those, apparently of more recent origin, lying near the endothelial surface contain no calcium salt. When we examine the aorta beyond the atheromatous patch, we lose any evidence of soap in the intima except in the deeper portions near the media, and only as the atheromatous area is approached do the layers between the fibrous strands become widened and the spaces become the site of deposit of soap granules. In the media, where, as we have described, the calcium salts are much more marked, similar clusters of granules

to those noted in the intima are found, with, however, the difference that in the media the clusters are compounds mainly of silver-nitrate salts—much calcium with a little soap,—while in the intima the proportion is exactly reversed.

To resume, it may be stated that by employing suitable methods it is found that calcareous deposits occur in the arterial walls, more particularly in the wall of the aorta, at an earlier age than is usually suspected.

These deposits vary as regards the region first affected. This variation may be in regard (i) to the internal elastic lamina, (ii) to the media, more particularly its muscle fibres, and (iii) to the intima in areas of nodose sclerosis.

Judging from the treatment of sections for the demonstration of fats and calcareous salts, the stages in this process are (i) degeneration of the affected region with deposit of fatty globules, (ii) formation of soaps, mainly with calcium, (iii) deposit of calcium salt devoid of a fatty moiety.

The results obtained in connection with calcified deposits in the arterial walls were confirmed and extended by an examination of other conditions of calcareous infiltration.

Calcareous Infiltration of Thickened Pleura.—The cases examined were evidently of some duration. There were thickened fibroid adhesions between the visceral and the parietal pleuræ, and in the centre of the fibroid layers dense calcified plaques occurred. The plaques were found to form rather irregular laminæ with somewhat irregular surfaces. By examining the material after decalcification, the section through what was a densely decalcified plaque was found to be stained a diffuse pale-yellow with Sudan III, no individual granules being present; but in the immediate neighborhood of the plaque was an aggregation of masses stained deeply with Sudan III. Therefore at the periphery where the process of calcification was still continuing there was definite evidence of the presence of fats.

In studying the decalcified sections it was noticed, as frequently observed by others, that the decalcified areas stained more deeply with hæmatoxylin than does normal tissue. Very

few nuclei were recognized. The matrix left behind after removing the calcium salts was of a hyaline nature. The existence of this matrix led Litten and others to the theory that the calcium salts are deposited as calcium albuminates. That the fat or soapy substances, still exist in connection with the matrix is indicated by the light-yellow color given with Sudan III; and remembering that, as we have noted, soap solutions and albumins form insoluble compounds with which calcium can combine, it would seem more probable that we are again dealing in this nstance with the resultant albuminous material deposited after the calcium has separated from the combination to unite with phosphoric and carbonic acids, and possibly to some extent also after a part of the soap has been split off to enter into more soluble combinations.

Calcified Fibromata.—A similar condition is found in calcified fibroma, in which the most extensive infiltration is not in the centre of the fibroid mass but in the outermost layer, whence it is proceeding towards the centre. In each of the specimens examined, three zones of change were well marked. In the outermost area (which varied in thickness), when the sections are stained with both Sudan III and silver-nitrate solution there is seen a dense black mass, the small amount of soap present in this zone being wholly obliterated by the black-stained lime salts. At the margin of this zone and farther inwards clusters of granules are to be seen, some of which stain with Sudan III, others with silver nitrate. At times an individual cluster, black at one end, yellow at the other, can be made out. In this area, between the groups of granules can still be seen the wavy fibres of connective tissue. This constitutes the second zone. The third zone, farther inwards, exhibits granules staining only with Sudan III. Thus there is well-marked fatty change, the presence of calcium soap, and finally the presence of calcium salts showing no soapy constituent.

That calcifying fibroid tumors should show a deposit of lime salts at the periphery is a phenomenon of the same nature as has been observed in connection with lithopedia and with kidneys undergoing calcification after complete cutting off of their blood

supply. The fibroid tumor that undergoes calcification is one that is undergoing necrosis through defective blood supply, and the calcareous salts deposited are not those already present in the tissue itself but those precipitated from the lymph which diffuses into the dead tissue from without. It follows that the diffusible salts are taken up by the first soapy substances with which they come into contact in this process of diffusion, and only when those in the peripheral zone become calcified do the soapy and fatty matters situated more deeply have the opportunity to form calcium compounds.

Calcareous Infiltration of Ovarian and Broad Ligament Cysts.—Cysts of this nature, in which a thin shell of lime salts is laid down in the fibrous tissues of the walls, are occasionally encountered. The process is usually confined to a layer bordering on the cyst cavity beneath the lining membrane of the cyst in the surrounding fibrous tissue. In these we have found the usual evidence of fatty change, of soap formation in the immediate neighborhood of the areas, and of deposition of lime salts.

Calcareous Tubercular Nodules.—The nodules can be decalcified, cut by the freezing microtome, and the sections treated with Sudan III or Scharlach R. It has been usual of late years to speak of tubercular caseation as a process of protoplasmic degeneration in which there is very little associated fatty degeneration. It is interesting therefore to note that by employing the methods described by us it can be demonstrated that the caseous areas which have undergone calcification contain a very considerable amount of fatty matter, so much indeed that the yellow staining areas of the tubercular nodule stand out very prominently from the surrounding tissue. Treatment of such sections with ether removes some of the material stainable with Sudan III, but by no means all. As might be expected from what has already been said, differences are to be made out between old and more recent calcified tubercular areas. In dense, old, calcified glands and nodules there is less of the fatty staining material left, and it is only here and there, where the soap moiety has not been wholly removed, that the deposition of the lime salts gives any results with Sudan III, while in softer and less

dense nodules larger and smaller granules staining with Sudan III are abundant. It is noticeable that very little fat is to be made out in the enveloping fibrous tissue. The question naturally arises, Where does this fat come from? Examination of lung tissue in cases of active tuberculosis showed that the fat and its products are already present in tubercles showing the early stages of central necrosis which as yet scarce show caseation. In caseous tubercles this fatty matter is still more evident, and in these also calcareous salts in the form of fine granules are to be detected. It was only in those tubercles in which the fatty substances were aggregated into larger granules that lime salts could be demonstrated. In the earlier non-caseous areas there was no reaction of the calcium; and it is to be noted that there were more Sudan-III-staining granules in the centre of the caseous areas than at the periphery. This series of events indicates the following stages, viz: necrosis, fatty degeneration, formation of calcium soaps, and deposition of calcium salts having no soapy moiety.

Pancreatic Fat Necrosis.—It has been pointed out by various observers that in pancreatic lesions followed by fat necrosis the neutral fats within the fat cells undergo decomposition, and as a result fatty acid crystals are left behind. These fatty crystals after a time unite with the lime salts of the tissue fluids to form insoluble calcium soaps. We found that in the experimental production of fat necrosis the fatty acid crystals could be stained readily enough, but unless the animals experimented upon remained alive for more than a week the lime salts were not to be demonstrated microscopically in the lesions. In other words, the combination of the fatty acids with calcium to form calcium soaps is not an immediate but rather a slow process. A definite period of time seems to be necessary before the calcium soaps accumulate so as to be present in stainable quantities. We examined one specimen of hæmorrhagic pancreatitis in man and found that fatty acid crystals had been deposited in the peripheral zone of the necrotic area. In this zone calcium salts were seen in small amounts distributed as a fine dust. The specimen, however, was an accidental find in a recent autopsy,

two small foci of necrosis in the tail of the pancreas, each scarce one centimetre across, being present, and there was no clinical indication of the length of time that the condition had existed. The earlier observations upon the presence of lime soaps in connection with pancreatic fat necrosis will be referred to later.

The preceding section of this paper may be reviewed as follows:

(1) All calcareous infiltrations are preceded by fatty changes and in them substances are found consisting of neutral fats, fatty acids, and soaps, which stain with Sudan III.

(2) By employing appropriate methods, the microscopic fat and fatty acids can be removed, the soaps remaining behind, the last being detected by reason of their differential staining with Sudan III.

(3) The granules in certain regions which give the soap reaction with Sudan III give also the calcium reaction with silver nitrate.

(4) As the process of calcification advances, many of the masses deposited in the part no longer stain with Sudan III, but only react for calcium salts.

In areas undergoing calcification three zones are to be made out: (1) The oldest zone of complete calcification, exhibiting dense calcareous deposit lying in a hyaline matrix; in this zone little soap or fatty substance is to be demonstrated. (2) The intermediate zone, in which granules of lime salts and soap granules are in close apposition; some of these granules give both the calcium and the soap reaction. (3) The most recent zone, which gives the reaction for fat, fatty acids, and soaps and fails to reveal calcium salts in sufficient quantities to be recognized under the microscope.

ON THE PRESENCE OF SOAPS IN THE ORGANISM UNDER PHYSIOLOGICAL CONDITIONS.

Since the observations above detailed indicate that soaps play a most important part in the process of pathological calcareous change, it appears desirable to compare with them observations

made upon the part played by the soaps in the physiology of the animal economy.

In 1857 Marcet stated that during fat digestion the fats are converted into soaps in the intestinal canal and these soaps are absorbed by the intestinal mucosa. The absence of any amount of soaps in the blood comparable with the amount of fat absorbed led others to conclude that the soaps as such did not pass beyond the endothelial cells, and to suppose that a glycerin molecule is furnished by these cells, the reconstructed fat being passed into the lacteals. Heidenhain pointed out that some at least of the fat is taken up by the leucocytes on the surface of the intestine and is carried by them directly into the lacteals and blood-vessels. More recently, Ramond has denied the validity of Heidenhain's observations, and has concluded that the greatest part of the fat absorbed enters the portal system and not the lacteals, and enters in the form of soap and not of fat. From this statement we infer that Ramond regards the soap as becoming transformed into fat during its passage from the endothelial cells to the portal capillaries. He finds also that the liver tissue contains a ferment capable of splitting fats into their respective fatty acids. Beneke and later Hoppe-Seyler have pointed out that the blood normally contains soap in solution. Beneke makes an interesting suggestion as to the manner of recovery from pulmonary fat embolism. He points out that possibly a solution of the fat of the emboli is brought about through the action of the lipase present in the blood, which converts the fat into a soluble soap. He also states that fat inoculated subcutaneously disappears so rapidly that the only satisfactory explanation is one based on its conversion into soluble soap.

Still more recently Schultz, who studied fats as a whole in the blood, found that no less than 28 per cent. of the total fatty acid obtainable is present in combination as soap. During starvation, when the fat deposits are used up, he found an increase of the fat in the blood and concluded that the fat is transported from its normal deposits by way of the blood. The fat in the tissue cells he holds becomes transformed into soap and thus passes in solution into the blood.

As to the manner of conversion of the fats into the fatty acid, in which form combination occurs with alkalies to produce soaps, we have the observations of Hanriot. This observer found that blood serum, pancreatic juice, and liver juice contain a ferment capable of converting the neutral fats into their respective fatty acids. This ferment was not present in muscle tissue, testicle, or thyroid gland. The substance isolated by Hanriot seemed by its action to be a true ferment, and was present in all the animals examined. He made a distinction between pancreatic lipase and serum lipase, claiming that they are different substances chemically. Cohnstein and Michaels, who also found a lipolytic ferment in the blood, differ from the former observer in believing that it is present in the red blood cells rather than in the serum.

There can be little doubt that the leucocytes and endothelial cells play parts in the synthesis of fat. It is well known that leucocytes are fat-carriers and that they transport fat from the intestinal canal during fat digestion. The quantity of fat transported must be very small indeed, but Arnold has found that subcutaneous injection of soap solutions into the backs of frogs attracts leucocytes which become filled with fat, and he also noted that eosinophilic leucocytes are especially active in this fat synthesis. Fischler has shown by similar experiments that sub cutaneous injections of soap are followed by deposit of fat in the organs, and that when a soap solution is passed through the renal vessels the fat particles can be seen between the endothelial cells and in the kidney tissue. Hence it would seem that endothelial cells take part in the conversion of soap into fat. This view is also supported by the statements of Ramond, namely, that the fat from the intestine passes through the intestinal mucosa, as through a dialyzing membrane, in the form of soap and is reconstructed on entering the portal system.

Munk studied the toxicity of soap and pointed out that soap solutions injected into the portal vascular system are only one-sixth as toxic as when inoculated into the peripheral vessels. From this it would seem that the portal endothelia or the liver parenchyma are especially active in converting the soaps into neutral fats.

THE PRESENCE OF SOAPS IN THE ORGANISM UNDER PATHOLOGICAL CONDITIONS.

It is not a little remarkable that very few observations upon the part played by soaps in pathological conditions have appeared in the literature. We have encountered isolated notes only upon the subject, and do not know a single research, properly so-called, in which their rôle has been fully worked out. Many years ago Virchow called attention to the presence of calcium salts in degenerating lipomata, and very correctly concluded that the fats present in these tumors combine with the calcium salts to form soaps. Kyber in 1880 referred incidentally to the part which both alkalies and fatty acids must play in the process of calcification. Somewhat later Jaeckle and others enunciated the theory that the formation of soaps is one step in the process of calcification of fatty tumors. But this theory has to our knowledge never been tested experimentally and would seem to have become obsolete. In 1890 Langerhans pointed out that in fat necrosis following pancreatic lesions crystals of fatty acids appear, and in many cases unite with calcium of the blood to form an insoluble calcium stearate which occurs at the site of the lesion. It does not appear that Langerhans ever continued these observations or expanded his findings so to apply them to calcification in general. Quite recently Fischler has published a preliminary note upon certain methods for detecting the presence of fats and soaps in the tissues; and though he does not state it definitely, he nevertheless seems to imply that his observations point to an important part played by soaps in more than one process.[1]

As indicated in the preceding pages, our observations all point to the fact that the formation of soaps is a necessary step in the development of pathological calcification. We may at this

[1] The number of the *Centralblatt* containing Fischler's article reached America, I may add, after I had forwarded to Philadelphia the abstract of my preliminary communication upon this subject, which was read at the meeting of the Association of American Physiologists in December, 1904. A study of Fischler's paper will show that he has reached his conclusions by the employment of methods differing from those given here.

point present our further studies on the chemistry of this process.

The analysis of aortas which were in the process of calcification yielded calcium soaps in small quantities together with sodium and potassium soaps, though chemical extraction did not give as clear an idea of the process as was obtained by treating sections with petroleum ether and later with Sudan III. Sections so treated seemed to indicate the presence of a fair amount of soaps. By analysis the quantities obtained were very small. Indeed the Sudan III stain after treatment seemed to show clearly that substances which *in vitro* are soluble are relatively insoluble when present in the tissues. For instance, sodium and potassium soaps are, as we know from every-day experience, easily soluble substances, while, on the contrary, calcium soaps are relatively insoluble. We were prepared, therefore, to find that the tissues contain relatively small amounts of the former, and large amounts of the latter, supposing that when formed locally sodium and potassium soaps would diffuse into the blood and lymph and so escape to a very large extent; but to our surprise the sodium and potassium soaps were not nearly so soluble in tissues as they are *in vitro*—in fact they are relatively insoluble in the former location.

ON THE EXISTENCE OF A COMPOUND OF SOAPS WITH ALBUMEN.

What would seem clearly to be the explanation of this difference was discovered in conducting observations in the test tube. Doubtless the reaction which we are about to describe has been noted before, but, if so, it has failed to come to our attention. If to a weak solution of a pure soap—sodium stearate for example, though the same is true of the oleate and the palmitate—there be added dilute egg albumen, a white rather flocculent precipitate forms. The reaction is not immediate: at the temperature of the room it requires half an hour or longer before it is complete; the precipitate is a combination of soap and albumen. The compound is slightly soluble in water and less soluble in alcohol. Under the microscope the precipitate appears granular, and when the particles are treated with Sudan III they assume the

identical pinkish-yellow color of the soap granules observed in tissues undergoing calcification.[1]

It is worth noting that the precipitate becomes denser and more pronounced when carbon dioxide is passed through the solution. Acid calcium phosphate may be added to a solution of egg albumen without precipitation; but if the salt be added to a combination of weak soap solution and egg albumen, made as above, precipitation takes place rapidly and yields a dense sediment.

The Chemical Isolation of Soaps from Pus.—We have already stated that we have been able to isolate *soaps* from more than one order of calcified tissue. We were able to isolate a material soluble in absolute alcohol, insoluble in petroleum ether, and precipitable on the addition of acid calcium phosphate from a number of calcified tuberculous glands of the mesentery. On the addition of a mineral acid the fatty acid was isolated from this combination and gave the characteristic stain with Sudan III.

Particular attention is called to the fact that similar soaps may be present in pus. The cases from which the pus was examined were, it is true, of chronic nature; and no opportunity has yet come to us for the study of large quantities of pus of more acute origin. With the pus of two tubercular abscesses (one an empyema, the other a case of Pott's disease), each yielding fifty cubic centimetres, tests were made. Each quantity was extracted with warm absolute alcohol for seventy-two hours, after which the mixture was filtered and the filtrate evaporated to complete dryness. The residue consisted of fat drops mixed with a white granular substance. The residue was collected and washed with ether and petroleum ether for four days, after which no fat or fatty acid could be demonstrated in the filtrate. It was now digested with warm distilled water, and on the addition of

[1] Mr. O. R. Mabie, Phm.B., has undertaken a study of this soap-albumen compound. His studies are at the present incomplete, but he has found that solutions of albumen when mixed with known strengths of chemically pure soap solutions absorb a certain quantity of the soap. He has used albumen from different sources, and with all he has obtained similar results, although different degrees of absorption have resulted.

calcium chloride to a quantity of the watery extract a white precipitate, stainable after evaporation on a watch glass with Sudan III, was obtained. In the unstained condition the precipitate appeared as a white fine powder. Fatty acids were obtained from the residue by adding acid, and the addition of hydrochloric acid to the aqueous solution caused an opalescence in the test tube. Sodium and potassium were detected in the residue by the flame, but no calcium was found. Fatty acids could also be detected and saponified from the extract in the petroleum ether.

ON THE EXPERIMENTAL PRODUCTION OF CALCIFICATION.

In Capsules Containing Fats and Fatty Acids.—Early in the course of this research experiments were undertaken to determine the organic compounds with which calcium salts may unite. Various products obtained as pure soaps were placed in celloidin capsules prepared by the method recommended by McCrae and introduced into the peritoneal cavity of rabbits where they were left for several days. The results of these experiments led to the study of the rôle of fatty acids and soaps in the process of calcification.

Von Kossa places the normal calcium strength of the blood of rabbits as varying from 1.06 to 2.01 per cent., which may be taken as about the limits of range. In our experiments, two celloidin capsules were prepared containing one gramme of sodium stearate and one gramme of sodium palmitate respectively. These were inserted into the peritoneal cavity of a rabbit and allowed to remain there eleven days. On removal, the contents of the capsule were more or less caked and contained some serum. The contents were treated with hydrochloric acid and precipitated with ammonium oxalate, and the solution then rendered alkaline with ammonia. The precipitate was collected and the calcium determined in the ash obtained on ignition. The sodium stearate was found to have combined with the greater quantity of calcium, there being 4 per cent. of the alkaline earth present. The capsule containing the sodium palmitate was found to contain 3.8 per cent. of calcium. Hence it was found that even in the short space of eleven days a definite

conversion of the sodium and potassium soaps into those containing calcium had taken place. Other experiments were carried out in which stearic and palmitic acids were substituted for their salts. The results were similar to the first, there being a decided increase in the calcium strength over that present in the serum of the animal.

The Experimental Production of Calcareous Infiltration in the Kidney.—All cells use fat in some form in carrying out their normal physiological function, and, as has already been pointed out, a great deal of fat is brought to them as soap. When the cell is injured, the soap it would seem is not metamorphosed but remains fixed in the cell in combination with the cytoplasm. This form of union is indicated by experiments on the kidney epithelium in animals, for the main conclusions to be drawn from Litten's experiments are that the tissue cells undergoing calcification are in a degenerating state and have already lost, or are in process of losing, their vitality. He further pointed out that calcification proceeds more rapidly if along with the dead and dying cells there should remain a good circulation to supply the calcium and other materials which are eventually deposited as a permanent precipitate. As Litten examined his sections only with hæmatoxylin staining he found no evidence of fatty products laid down in the areas of his "coagulation necrosis."

The animal experiments reported by Litten were the first dealing with the question of the calcification of the renal tubules. They demonstrated that, if the renal vessels were ligated for two or more hours and then loosened, after eight or ten days crystals of lime salts were to be found in the cells of the convoluted tubules chiefly. The better the blood supply to the organ after ligation, the more quickly did the calcification proceed. The explanation offered was as follows: arrest of the blood to the kidney having caused "coagulation necrosis" of the tubular epithelium, the later advent of the calcium-containing tissue fluids brought about precipitation in the tubules of "calcium albuminate." Although this theory of Litten has been current for twenty years, it is noteworthy that the production of calcium albuminate had not been demonstrated *in vitro*.

We must, however, conclude that in the cells which are injured there is a change in the protoplasm, and of this there is adequate evidence found in the fact that the nuclei stain poorly and the cell substance appears swollen and homogeneous. The alteration of the nucleus is perhaps the chief indication that the cell has suffered grave metabolic changes. There is no evidence at this point of the process that the cell substance is diminished in amount—indeed, on the contrary, the actual amount of cell material appears increased. This is in keeping with the recent observations which point to the conclusion that fat appearing in the cell is not the product of cytoplasmic decomposition, but is absorbed from without. The view we wish to emphasize is that in the degenerating cells absorption of material from without still continues whether this material be albuminous or fatty. But owing to nuclear disturbance, or more broadly to lowered metabolic activity, the substances so absorbed cannot be used up; they no longer become combined into the cell cytoplasm or bioplasm proper, and remaining in an uncombined state may now become subject to various pathological changes.

Neuberger has shown that a number of chemical irritants produce the same effect on the kidney as ligating the renal vessels, and that they lead to calcification of the same sets of tubules. We repeated Neuberger's experiments, using the salts of the heavier metals, mercury, copper, lead, etc., with the result of producing calcareous degeneration in the convoluted tubules of the cortex of the kidney. The rabbits were inoculated subcutaneously with solutions of the soluble salts of these metals and then killed after ten days. A small portion of the kidney was removed for microscopical purposes. Frozen sections were stained with Sudan III and silver nitrate and both gave satisfactory pictures. In consecutive sections it could be shown that the degenerated tubules which reacted for the fatty substances with Sudan III also reacted with silver nitrate; and, moreover, that when a section was treated first with Sudan III and after staining was subjected to silver-nitrate solution, all the Sudan-III-staining tubules were covered over by the intense black stain of the silver salt. That is, the identical tubules which

were shown to contain a fatty-staining substance also contained calcium salt (vide Figs. 1 and 2, Plate xxx). The calcium could be wholly removed from the tissue by the use of dilute acids without affecting its fatty-staining qualities. If frozen sections were treated first with seventy per cent. alcohol and later with petroleum ether, it was still possible to find the tubules picked out with Sudan III and silver nitrate. Finally it has been possible to extract from the cortex of the kidney, potassium, sodium, and calcium soaps. These results indicate that the formation of a calcium soap is one stage in the process of calcification in the kidney; that, degeneration and death of the cells being effected, the cells are not able to utilize the potassium and sodium soap brought by the blood, and hence these are precipitated in combination with albumens, and later the calcium from the tissue fluid and blood is fixed in an insoluble state by this compound. While we agree with Litten, Neuberger, von Kossa, and others, that necrobiosis, improperly referred to by them as a "coagulation necrosis," occurs, we look upon this as only one stage in the whole process of calcification.

Von Werra believes that such degenerated cells of the kidney may return to a normal condition. We can hardly accept this view, as calcareous degeneration of cells is the last stage in a pathological process in which the cells and nuclei are disintegrated. Hence it is improbable that they can regain their vitality.

Tartarini-Galleani claims to have studied the kidneys of rabbits after injecting bichloride of mercury into the substance of the organ. He states that the lesion produced is a tissue destruction followed by fibrinous coagulation, which after the ninth day becomes impregnated with calcium salts. The calcification in the kidney remains circumscribed to the area of the organ directly affected by the injection, and he holds that it is neither preceded nor accompanied by the phenomenon of fatty change. We have repeated his experiments in two rabbits and in each the result was different from that described by him.

Sections of the inoculated kidney treated with Sudan III showed the cells of the convoluted tubules to be swollen and

filled with fatty granules. The tubules were well outlined from the surrounding tissue by reason of the stained fat in the cell contents. The glomeruli and the interstitial tissue appeared unchanged, while the arterioles of the cortex showed Sudan-III-stained granules in their walls. These granules were distributed more loosely than was seen in the epithelium. The affected convoluted tubules presented collections of irregular granules staining not so intensely as neutral fat, but rather a brownish-yellow. The tubules in the pyramids showed a striking difference. While they were less affected than in the cortex the condition of the fat in them differed from that of the convoluted tubules. The individual cells showed no change in size or shape, and the nuclei retained their staining power. However, the Sudan III picked out numerous fine globules situated towards the inner margins of the cells—that is, towards the lumen and away from the nucleus. In some cases it appeared that the fatty globules were in the process of being extruded into the lumen. The Sudan III granulation noted in the straight tubules was of the character of neutral fat. No calcium deposit could be demonstrated in the straight collecting tubules, while in the cortex the convoluted tubules which showed the Sudan III granulations reacted to silver nitrate in the manner of calcium salts.

Résumé.—Our studies upon experimental calcification in the kidney confirm and extend the observations made upon calcareous changes in human tissues. If the tubules become the seat of the calcareous deposit it is possible to recognize the following stages, viz:

(1) A stage of cell degeneration characterized by swelling of the cell substance and diminution of the nuclear chromatin, the cells becoming swollen and homogeneous;

(2) A later stage in which fat appears in the cells, apparently having arisen by absorption.

(3) A final stage in which calcareous salts appear in the cells accompanied by soaps. An intimate relation between cytoplasmic degeneration and the formation of insoluble soaps would seem to exist. Judging from observations made in test tubes,

the relatively insoluble soaps are compounds between soap and certain albuminous matters liberated from the cytoplasm. The calcium from the tissue fluids and the blood becomes fixed by the albumen-soap compound.

Experimental Calcification of Muscle Fibres.—That muscle fibres may become the seat of calcareous deposits is well known. We inoculated a weak solution of bichloride of mercury into the quadriceps muscle of a rabbit, killing the animal at the end of eight days. On examination the muscle at the site of the inoculation showed extensive degeneration and softening. Individual fibres were swollen and in some places the sarcolemma was broken. Other swollen fibres showed band-like constriction. Still other muscle fibres were completely broken down and invaded by numerous polymorphonuclear leucocytes.

The degenerated muscle fibres proved an interesting study. A fine granulation not found in healthy fibres, and staining in Sudan III, occurred in their substance, besides which leucocytes loaded with fat globules invaded the degenerating fibres and lay in the interstitial tissue. The fatty-staining material within the leucocytes appeared in the form of large and small granules, but these did not coalesce to form large fat globules as is seen in adipose tissue.

The question arises as to whether the leucocytes are bringing the fat to the injured tissue or taking it away. We are inclined to the latter view, for among the degenerated fibres were some in which the sarcolemma was intact and which were free from leucocytic infiltration, but which nevertheless contained granules staining with Sudan III. Moreover, the leucocytes at the margin of the injured tissue contained no fatty-staining material.

We think it probable that this process is not unlike that described in the kidney, namely, a state of lowered vitality of the individual cells through which, being no longer able to deal with the fatty material brought to them in solution, they form with it a relatively insoluble compound in the cytoplasm of the degenerating cells.

Experimental Fat Necrosis.—In order to investigate further the changes which fatty products undergo in the body, we pro-

duced fat necrosis in a rabbit by the intraperitoneal inoculation of fresh extract of pancreas. The injection was repeated on the second day and the animal killed on the fifth day. At the post-mortem a few tags of fat necrosis about the size of a pea were found scattered in the mesentery and the posterior abdominal wall. Frozen sections of these showed that the fat did not exist in the large distended fat cells but that the globules were replaced by numerous fine crystalline particles staining with Sudan III. On treating the sections with silver nitrate, the subperitoneal margins of the fat necrosis showed the presence of a slight amount of calcium salts appearing like a fine dust-like deposit. All the areas of fat necrosis did not show the presence of calcium with silver nitrate

As already stated, Langerhans showed in 1890 that calcium salts appear in the areas of fat necrosis. He describes the process as one in which the fat is converted by ferment action into fatty acid. The calcium salts of the blood then become attracted to the fatty-acid moiety and form a calcium soap.

ON THE SOURCE OF THE FAT IN THE DEGENERATED CELLS.

Before concluding, it is, we think, essential to indicate our views regarding the source of the fat present thus in all cells that undergo calcification and essential for the due development of the process. To discuss the matter adequately would demand that the numerous recent studies upon fatty degeneration be passed in review: such full discussion would carry us too far afield. It must suffice that we call to mind the reasonable deductions from the work of recent observers which appear to bear immediately upon our present problem.

We may, I think, in the first place safely accept that fats are absorbed by the cell from the surrounding medium in the form of diffusible soaps. Taken up by the cell these soaps may either be reconverted into neutral fats and stored as such in the cytoplasm, or may undergo assimilation proper, becoming part and parcel of the cell substance. That such assimilation does occur with associated loss of individuality on the part of the fats is,

we hold, demonstrated by sundry recent observations. Thus Rosenfeld and others have shown that kidneys presenting no fat recognizable by microchemical means will, nevertheless, yield upon careful analysis as much as fifteen per cent. of fats; and Doremeyer, that mere extraction by ether will not liberate the greater part of tissue fats: the cells have to be digested with pepsin before all can be isolated; a clear indication that the fats have previously been in combination with proteid substances in the cells.

If, then, these possibilities be accepted, it follows that the appearance of fats and soaps in a degenerating cell may be from at least two sources, namely:

(1) The absorption of soaps from the circulating lymph and blood, the cell still retaining the power of splitting off the fat, but being unable to utilize this in metabolism, so that it becomes stored up in a non-diffusible form.

(2) Disintegration of the cell substances so that the fats become liberated from their combination with the cytoplasm and appear in the form of discrete particles, or globules, of neutral fat, which now reveal their presence by reacting with Sudan III.

A third possibility has to be kept in mind, namely, that soaps may be absorbed from the blood and lymph by cells with vitality so depressed that no conversion takes place of the absorbed soap into neutral fat, but combining with the disintegrated albuminous molecules this forms a relatively insoluble soap-albumen compound.

Into the controversy regarding the formation of fat by the decomposition of the albuminous molecule I will not enter, save by suggesting that the opposing views may possibly be harmonized through the demonstration here afforded, that albumens form a combination with soaps. The increase in fats determined in tissue autolysis by Waldvogel, Hildesheim and Leathes, Landsteiner and Mucha, and others is, it may be, to be explained, not as due to the appearance of a decomposition product of the albumen molecule proper, but as brought about by dissociation of this albumen-soap compound.

The time is not ripe for, neither do our personal observations

up to the present permit, any discussion of the part played by cell ferments in any of these processes.

As to which of the above alternatives—absorption or dissociation—is the more frequently in action as a precursor of calcification, we would point out that when the cell becomes distended with fatty globules (and this is common in atheromatous areas) we must conclude that absorption from without is in the main responsible for the condition.

CONCLUSIONS.

It will be seen from the above that we have studied the conditions associated with the deposit of calcareous salts: (1) in connection with normal and pathological ossification, and (2) in pathological calcification as exhibited in (a) atheroma of the vessels; (b) calcification of caseating tubercular lesions; (c) calcification of inflammatory new growth, and (d) degenerating tumors; and we have induced experimentally deposits of calcareous salts in the lower animals: (a) within celloidin capsules containing fats and soaps; (b) in the kidney, and (c) in connection with fat necrosis.

I. We have found that bone formation and pathological calcareous infiltration are wholly distinct processes. In the former there is no evidence of associated fatty change, and the cells associated with the process of deposition of calcium are functionally active. In the latter there is an antecedent fatty change in the affected areas, and the cells involved present constant evidences of degeneration. The view that would seem to account best for the changes observed in the latter case is that with lowered vitality the cells are unable to utilize the products brought to them by the blood, or which they continue to absorb, so that the normal series of decompositions associated with their metabolism fails to take place and hence they interact among themselves in the cytoplasm with the result that insoluble compounds replace soluble ones.

II. Besides the fact that calcification is always preceded by fatty change within the cells, another fact should be emphasized,

namely: that combination of the fats present with calcium salts to form calcium soaps tends to occur. The stages immediately preceding these are difficult to follow with anything approaching certainty, perhaps because the earlier stages vary under different conditions. In fat necrosis, for instance, the cells affected are normally storehouses for neutral fats, and as long as they remain healthy neutral fats alone are present in them. When they are subjected to the action of the pancreatic juice with its fat-splitting ferment the cells are killed and coincidently the neutral fats are decomposed, fatty acids being deposited. The fatty acids now slowly combine with the calcium salts. In degenerating lipomata the process would seem to be similar.

But in other cases the cells are not obviously fat-containing in the normal state; nevertheless prior to calcification they undergo so-called fatty degeneration, which is really a form of cell degeneration accompanied by fat infiltration. As regards the source of the cell fats in general we may safely accept:

1. That fats are transported in the blood as diffusible soaps.
2. That taken up by the cells these soaps may either—
   (a) Be reconverted into neutral fats and become stored in the cytoplasm as such, or
   (b) undergo assimilation proper, becoming part and parcel of the cell substance, in which case they are not recognizable by the ordinary microchemical tests.
3. If these two possibilities be accepted it follows that the appearance of fats and soaps in the degenerating cell may be due to either—
   (a) Absorption or infiltration of soaps from the surrounding medium, the degenerating cell retaining the power of splitting off the fat but being unable to utilize this in metabolism.
   (b) Cytoplasmic disintegration with dissociation of the soap-albumen combination or, more broadly, liberation of the fats from their combination with the cytoplasm.

The appearances seen in the cells of atheromatous areas indicate that the first of these does occur.

III. In areas undergoing calcareous infiltration we have demonstrated the presence of soaps, and this often in such quantities that they can be isolated and estimated by gross chemical methods. By microchemical methods also we have been able to show that after removing all the neutral fats and fatty acids by petroleum ether there remains behind a substance giving with Sudan III the reaction we associate with the presence of soap. And experimentally we have produced these soaps within the organism, more particularly by placing capsules containing fats and fatty acids within the tissues and after several days finding that the capsules contain calcium soaps and possess a calcium content far in excess of that of the normal blood and lymph.

IV. While these are the facts, certain of the details of this reaction demand elucidation. The existence of sodium and it may be potassium soaps in the degenerated cells is comprehensible if we accept that these are present in the circulating lymph and simply undergoing absorption. But even then, as these are diffusible substances how is it to be explained that they become stored up in these particular areas?

We have found that, as a matter of fact, in regions which give the reaction for soaps, but which give no reaction for calcium (which therefore presumably contain at most amounts of the insoluble calcium soap too small to need consideration), the ordinary solvents for potassium and sodium soaps do not forthwith remove the stainable material; they are relatively insoluble.

The reason for this insolubility is suggested by the observations made in the test tube, that soap solutions mixed with solutions of white of egg or blood serum form a precipitate of combined soap and albumen,which likewise is insoluble in water and alcohol. The indications are therefore that in cells undergoing degeneration, with degeneration of the cytoplasm, certain albuminous molecules unite with the soaps present to form relatively insoluble soap-albuminate.

V. With regard to calcium soaps, these are also present and in certain stages appear to be the dominating form in the affected tissues. Two questions suggest themselves, viz.: what is the source of calcium, and what is the process by which they become formed?

As to the source, the amount present in well-marked calcification is far in excess of the normal calcium contents of the affected tissue. If in the kidneys of experimental calcification three hundred times as much calcium may be present as in the normal kidney (von Kossa), the calcium must be conveyed to the part by the blood and lymph, and that this is so is demonstrated, as we have pointed out, by the distribution of the infiltration in solid organs, that like ovarian fibroids have undergone necrosis, in which the earliest deposits are superficial.

As to the process, there are three possibilities:

1. That sodium and potassium soaps and soap albuminates are first formed and that interaction occurs between them and the diffused calcium salts from the lymph, the less soluble-calcium replacing the sodium and potassium.

2. That under certain conditions the calcium salts act directly on the neutral fats present in the degenerating cells.

3. That the neutral fats are first broken down into fatty acids and that these react with the calcium salts to form the soaps.

We are assured that the first process occurs and that because in the boundary zone of areas of calcification we can detect soapy particles devoid of calcium, identical in position and arrangement with the particles more deeply placed which give the calcium reactions. But this is not the only reaction. In case of fat necrosis we see clearly that the third process is in evidence. And we are far from being convinced that the second does not also obtain. We have been impressed by the large accumulation of neutral fats in the cells in cases of early atheroma and the absence at any stage of the process of recognizable fatty acid. While soaps, it is true, are compounds of fatty acids with alkalies, it is recognized in ordinary domestic life that they can be formed by the direct action of strong lye upon ordinary fats, and this even in the cold. It is quite possible therefore that there occurs a similar direct process in the organism.

The point is worth noting, however, that this does not occur in healthy cells the seat of fatty infiltration. We therefore leave this an open question, only laying down that, as indicated by

the hyalin albuminous matrix left when calcium salts are dissolved out of an area of calcification, there must exist a calcium soap- or fat-albuminate similar to the potassium and sodium soap-albuminates already mentioned—such an albuminate as we can form with calcium soaps in the test tube.

VI. In old areas of calcification soaps are largely if not entirely wanting, although these are to be detected at the periphery, when the process is still advancing. The reactions given by these older areas are almost entirely those of calcium phosphate, though some calcium carbonate is at times to be made out.

This seems surely to indicate that the final stage in calcification is an interaction between the calcium soap-albuminates and substances containing phosphoric and carbonic acids. Such substances, it is needless to say, are present in considerable amounts in the lymph and blood. We must conclude that the acid sodium phosphates of the lymph act on the calcium soap, the highly insoluble calcium phosphates being formed (plus the albuminous moiety of the original compound) and diffusible sodium soap being liberated, while similarly alkaline carbonates form calcium carbonate and liberate sodium and potassium soaps. Calcium phosphate and calcium carbonate thus become the insoluble earthy salts of old crystalline areas of calcification.

VII. As already stated very little soap is to be found in these old areas. It is possibly worth suggestion that the soaps liberated in this last reaction, as they diffuse out, again react with diffusible calcium salts and form calcium soaps which once more react with the alkaline salts to produce the phosphates and carbonates; that, in short, they have a katalytic action. Certain it is that old calcareous areas are extraordinarily dense, and have a coarse crystalline structure, wholly at variance with the finely granular appearance of the more recent areas, and these large crystalline masses, it would seem, can only be formed by successive deposition of new material and eventual fusion, as the interspaces become filled in between the original masses.

In conclusion I wish to thank Professor R. F. Ruttan of McGill University and Mr. J. R. Roebuck, B.A., for their kind assistance in carrying out some of the chemical analyses, and Drs. M. E.

Abbott and F. A. J. Lockhart for specimens of calcareous degeneration of tumors.

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FIG. 1.

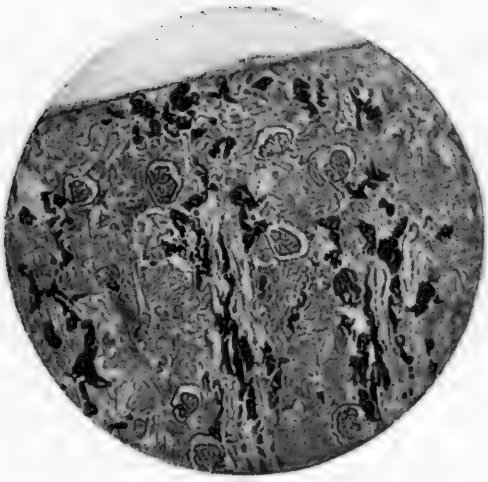

FIG. 2.

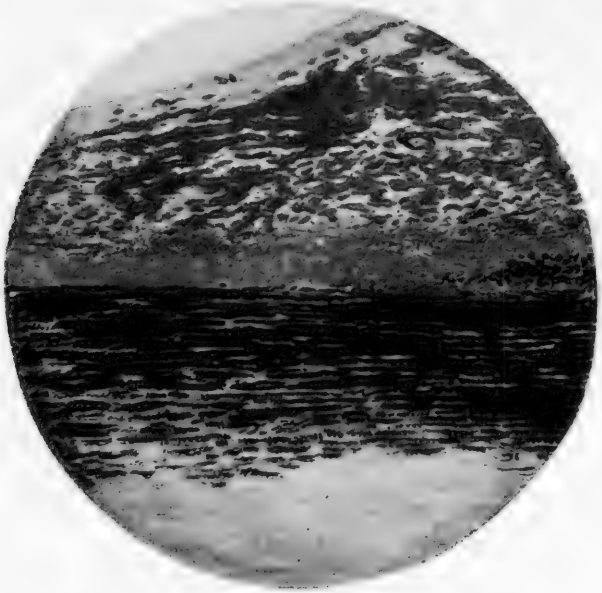

FIG. 3.

FIG. 4.

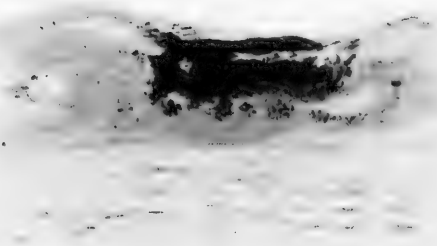

FIG. 5.

DESCRIPTION OF PLATES.

Plate XXX.

Fig. 1.—Kidney from rabbit which died eight days after inoculation with corrosive sublimate. Frozen section stained with Sudan III showing the fatty condition of certain convoluted tubules.

Fig. 2.—Section from the same kidney as Fig. 1, stained with silver nitrate followed by weak saffranin. Similar convoluted tubules picked out by the silver deposit as seen in Fig. 1 with Sudan III.

Plate XXXI.

Fig. 3.—Aorta with large fat masses towards the lumen with similar lighter staining particles near the boundary zone between the intima and media. In the media the blackened deposit representing calcium salts is very intense and is arranged in parallel rows. These rows with higher magnification are seen to be composed of numerous small crystals. Among these rows of calcium salts are also seen the yellowish granules of Sudan III staining.

Fig. 4.—Section of aorta stained with Sudan III. The fatty plaque shows a lighter stained part in its middle, representing the area of soap formation and calcium deposit.

Fig. 5.—Same aortic plaque as seen in Fig. 4, treated with silver nitrate solution. The area which with Sudan III stained a light yellow is now represented as the black deposit, the site of the calcium salts.

ON THE PHYSIOLOGY OF HEART-BLOCK IN MAMMALS, WITH ESPECIAL REFERENCE TO THE CAUSATION OF STOKES-ADAMS DISEASE.

By JOSEPH ERLANGER.

(*From the Physiological Laboratory of the Johns Hopkins University.*)

PLATES XXXII–XXXV.

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Protocols of the experiments of Part II.

INTRODUCTION.

Through the kindness of Dr. Osler, to whom my thanks are due for interesting me in this subject, I have had the opportunity of making a prolonged series of observations on a typical instance of Stokes-Adams disease, and have been granted the privilege of using the hospital records of this case.

A preliminary report of the results of these observations was made at a meeting of the American Physiological Society on Dec. 28, 1904, and an abstract of the report was published in the Proceedings of the Society.[1] The studies led to the conclusion that the circulatory disturbances presented by this case owed their origin to the condition which is now generally termed heart-block. But it was found that many of the phenomena observed could not be explained upon the basis of recorded experimental data on heart-block. It was therefore decided to undertake an investigation of heart-block in mammals. A preliminary report of the results of this investigation has been published in the *Zentralblatt für Physiologie*.[2]

Upon comparing the results obtained from these studies of heart-block in man and in the dog with the results obtained in studies of typical instances of Stokes-Adams disease by other investigators, an obvious relation was found to exist between the two conditions. This relation was pointed out at a meeting of the Johns Hopkins Medical Society held on March 20, 1905, when a full report of the essential facts of this investigation was made.[3]

It is therefore obvious that these studies may be conveniently divided into three parts, namely:

I. Observations on an instance of heart-block in man.

II. On the physiology of heart-block in the dog.

III. On the relation of heart-block to Stokes-Adams disease.

[1] Erlanger, *American Jour. of Phys.*, 1905, xiii, xxvi.

[2] Erlanger, *Zent. für Phys.*, 1905, xix, 9.

[3] See abstract in *Bull. of the Johns Hopkins Hosp.*, 1905, xvi, 202.

PART I.

OBSERVATIONS ON AN INSTANCE OF HEART-BLOCK IN MAN.

HISTORY OF A CASE OF STOKES-ADAMS DISEASE.

The following is a brief abstract of the hospital records of the case of Stokes-Adams disease, the study of which forms the basis of the first part of this communication.

C. B., colored, aged thirty-four years, was admitted into the Medical Department of the Johns Hopkins Hospital on November 17, 1904, complaining of vertigo, soreness in the chest, and shortness of breath on exertion. In his personal history there is nothing of importance excepting a clear history of syphilitic infection in 1897. At this time he had "rheumatism" and an extensive skin eruption. In 1901 he was treated in the out-patient department for a costal gumma. The treatment was continued for but a short while. There is also a clear history of gonorrhœal infection.

The present illness began about two months before admission when, while unloading a lumber wagon, the patient was seized with an attack of dizziness. In this attack he did not fall. Excepting very slight attacks of dizziness which were brought on by a sudden turn, the patient had no severe attacks until November 11, when, upon suddenly turning, he was seized with an "attack of swimming in the head in which it seemed as though his breathing stopped." On November 12 he felt very dizzy but went to work. On this day while eating his dinner he fell from his chair unconscious and received a severe wound on the head. He believes that he was unconscious for two or three minutes. He had several similar attacks on that day, but has had none since.

When admitted into the hospital the patient stated that he felt dizzy, was short of breath, and had a peculiar dragging sensation in the chest.

The note dictated by Dr. Thayer at this time states in part: The patient is a healthy-looking man with mucous membranes of good color. The pulse at the wrist is eight to the quarter of a minute, but at the apex thirty-one beats to the quarter are heard. The radials are thickened and palpable.

There is well-marked heaving of the precordial area. The impulse seen in the sixth interspace is rather diffuse, and on palpation the heart is felt, though it is difficult to localize the outermost point. There is a very well-marked retraction in the sixth interspace in the mammillary line. At the outermost point, where the stronger beats are clearly seen and where the feebler ones are barely palpable, the sounds are clear; one strong beat is followed usually by two extremely feeble impulses the sounds of which are just heard. The intermediate sounds are associated with feeble but clear second sounds.

The liver reaches 10 cm. below the ensiform process. The veins of the neck are very full, especially on the right side. Waves are seen in these, the rapidity of which it is difficult to make out.

The first attack which was observed by the ward physician occurred on

December 22. It came on while the patient was walking. He fell without warning. There was no definite loss of consciousness, nor were there convulsions. A note made on January 23 states that "up to date the patient has had but five definite syncopal attacks in which he has lost consciousness or fallen, although he very often has spells of dizziness. During the attacks the pulse-rate is diminished and the veins are very much distended." On March 20 the patient had many attacks, but at this time it was noted that a steady increase in the heart-rate was in progress. On March 29 the heart was apparently normal; its rate was seventy-two per minute. The patient has been under continuous observation up to date (June 2); and since the last note was made the condition of the heart has been normal, and the patient has had no syncopal attacks.

THE METHODS EMPLOYED IN OBTAINING THE TRACINGS.

The tracings of the cardiac impulse were made with Marey's cardiograph. The apex beat of the patient was not well defined, and we, therefore, could not be certain that the button of the receiving tambour always occupied the same position. Furthermore, the precordial pulsation was so diffuse that the supports of the cardiogram could not be rested upon parts of the chest wall that were not moved by the beats of the heart. Therefore the position occupied by the supports of the tambour, as well as by the button of the tambour, must have had an influence upon the form of the cardiogram. Consequently the cardiograms obtained are by no means typical, but, so far as concerns the phenomena investigated, they have answered every requirement.

The tracings of the venous pulsations were obtained from the external jugular vein at a position about 4 cm. above the clavicle. The method of air transmission was used. The pulsations were received by a tambour 7 cm. in diameter upon the head of which was stretched very delicate rubber dam. The tambour was held in place by a rod which extended from a universal joint strapped to the head of the patient. The pelotte of the tambour made very light contact with the vein. We were particularly fortunate in finding it possible to take the pulsations from the external jugular vein, for this vein lies well without the region affected by pulsations transmitted from the carotid artery.

As some stress will be laid upon the form of the jugular pulse, it should be stated here that the apparatus employed was practically devoid of fling. When subjected, by artificial means, to

rates of movement similar to those developed while making the tracings, the lever recorded practically no inertia waves.

The pulsations in the brachial artery were recorded with the sphygmomanometer devised by the author.[4] As a preliminary to the registration of the pulse, the pressure was determined at which pulsations of the greatest amplitude were obtained: the tracings were made with this pressure on the arm. Delicate tracings may be obtained in this way with the greatest of facility but, on account of the great distance through which the lever was moved by the anacrotic rise of pressure, this part of the tracings is somewhat deformed by fling. The points of all of the levers were arranged to write upon the drum directly above one another. But nevertheless in analyzing the records similar positions of the various tracings were marked by the levers in the usual way. It is of course necessary to make a correction for the time lost in the transmission of the pulse-waves. For this purpose the rate of transmission of the pulse-wave in the brachio-radial artery was determined. It was found to be 7.6 m. per second. With this as a basis it has been estimated that the time lost in transmission of the pulse-waves to the point in the brachial artery from which the tracings were obtained was about three hundredths of a second, or somewhat less than one fourth of a division of the time record. The distance from the heart to the place on the external jugular vein from which the venous tracings were obtained was roughly one half of that distance in the case of the arteries. As the rate of transmission of waves in the veins is probably about one half of that in the arteries [5] the corrections to be made are approximately the same for both tracings.

For the sake of clearness the original tracings of Fig. 1 have been transcribed on to co-ordinate paper with a pantograph (Fig. 3). This copy will serve to illustrate in a general way the relations which the various features of the tracings bear to one another. It must however be borne in mind that the reproduction made with the pantograph is not as accurate as the original. Therefore the former cannot be used for interpreting time

[4] Erlanger, *Johns Hopkins Hosp. Reports*, 1904, xii, 53.
[5] Morrow, *Pflüger's Archiv*, 1900, lxxix, 442.

relations when the values involved are at all minute. The manner in which the reconstructions included in this figure were made will be described later.

In Figs. 1 and 2 the tracings are from above downward: cardiogram, arterial sphygmogram, and venous sphygmogram. The tracing of the jugular pulse in Fig. 2 is of no value. While it was being made, the lever was pressing so firmly against the drum that the finer details were completely obscured and the time relations disturbed. The record is reproduced here because the cardiogram and sphygmogram show certain features better than any other tracings we have made. Figs. 1 and 2 are practically continuous tracings; a brief delay was interposed between them only for the purpose of re-adjusting the receiving and recording instruments. On the time record one division equals one fifth of a second.

ANALYSIS OF TRACINGS OBTAINED DURING COMPLETE HEART-BLOCK.

The Proof that Complete Heart-block Existed.—In analyzing tracings in which the waves are so numerous and varied as in these, it is well to begin with a phase of the cycle which offers the least difficulty in interpretation. We therefore choose as a starting-point the large waves on the arterial sphygmogram (Figs. 1, 2, and 3) The foot (a) of such a wave corresponds, when the correction is made for the time lost in transmission,[6] with a point about half-way up the wave (a–f) on the cardiogram. Therefore the ascending limb (a-b) of this wave marks the beginning of a ventricular systole. The descending line (e–f) on the cardiogram undoubtedly represents the relaxation of the ventricles. The portion of the tracing which is included between these two lines and which, in perfect cardiograms, is the plateau stage of ventricular contraction, is rather irregular. In Fig. 2 two definite positive waves are to be seen on it.

On the tracing obtained from the jugular vein, this phase of the cardiac cycle is, as a rule, occupied by two large waves. The foot (a) of the ascending limb of the first wave corresponds,

[6] Without further notice this correction will be made wherever necessary.

with the exceptions to be mentioned later, with the beginning of ventricular systole. The foot of the ascending limb of the second wave (c) corresponds with the beginning of the second elevation in the cardiogram. The descending limb of the second wave reaches its lowest level (f) somewhat later than the line in the cardiogram which presumably marks the termination of ventricular relaxation. The foot (f) of the descending limb of the second large venous wave marks the lowest level in each cycle.

To complete the description of this phase of the cycle it becomes necessary to return to a consideration of the sphygmogram. But here we are met with some difficulty in interpretation because corresponding portions of the curve are deformed by fling. In Fig. 4 is reproduced another tracing of the brachial pulse obtained with the sphygmomanometer modified so as to do away with at least most of the fling. This was accomplished by replacing the compound lever of the instrument with a simple, light, aluminum lever.

The tracing obtained in this way shows that the primary wave is followed after a short pause by another wave, the apex of which is perhaps not quite as high as that of the first wave. It is possible that this wave corresponds in time with the wave (c–e) indistinctly seen in Figs. 1 and 2.

Two interpretations may be made of the parts of the tracings which have been described.

1. They represent the events of but a single ventricular systole. Then the secondary wave on the sphygmogram becomes a dicrotic wave, and the second wave on the jugular tracing becomes the so-called diastolic wave.[7] But upon this assumption there is no very satisfactory way of accounting for the second wave on the cardiogram. However, in view of the fact that the normal cardiogram is notoriously variable, this difficulty does not seriously weaken the conception that we have to do here with but a single ventricular systole.

2. The main ventricular systole is followed by an extra-systole. Upon such an assumption the secondary wave represents the effect produced by this extra-systole on the pressure in the

[7] Gerhardt, *Schmiedeberg's Archiv*, 1894, xxxiv, 402.

peripheral arteries, the first and second waves on the jugular tracing become systolic regurgitant waves, and the two elevations on the cardiogram represent the periods of rising tension of the two ventricular systoles.

We cannot determine positively which of these is the proper interpretation. The fact that as long as the heart-block was complete the same form of cardiogram was always obtained, is perhaps the most convincing evidence which can be offered in favor of the view that the portion of the curves described represents the events of a single ventricular systole. This interpretation is further supported by the fact that no heart-sounds corresponding with an extra-systole could be heard. Fortunately whatever the proper interpretation may be, it does not in any way modify the significant features of these tracings, to a consideration of which we shall now pass.

In Fig. 1 it will be seen that, following the depression which corresponds with ventricular relaxation, the cardiogram rises slowly and evenly (g–h–k) to the summit of a plateau. The principal markings on this plateau consist of more or less regular negative waves (k–l), two or three in number. The two limbs of the negative waves are capped by small positive waves (k–l). In addition, each plateau which is included between the negative waves shows slight undulations. The most constant and prominent of these (n) begins shortly after the termination of the small positive wave which caps the ascending limb of each negative wave. The corresponding portions of the cardiogram of Fig. 2 differ in some respects from those of Fig. 1. The terminations of the short plateaux are marked by much more distinct positive waves, the descending limbs of which pass directly into the negative waves.

On the sphygmogram, particularly that of Fig. 2, it is seen that a small positive wave is recorded synchronously with each of the positive waves which terminates the shorter plateaux on the cardiogram, and furthermore, that each positive wave on the sphygmogram is followed by a negative wave which is apparently synchronous with a corresponding negative wave on the cardiogram.

The tracing obtained from the jugular vein, as a rule, shows two waves for each of the shorter cycles on the cardiogram. The smaller of these waves (n) is synchronous with the most distinct undulation (n) on the plateaux: the larger wave (k) is synchronous with the positive wave (k) which terminates each plateau; and the deeper negative wave corresponds more or less exactly with the negative waves which separate the plateaux on the cardiogram.

I believe that each of the shorter cycles in the portions of the tracings under consideration represents an auricular systole and diastole. The systole occurs at the termination of the shorter plateaux and produces the positive wave at this place.

The Reconstructed Cardiogram.—It can be shown that the short cycles on the cardiogram, which we have assumed to be auricular cycles, are not limited to the periods included between the ventricular systoles, but that they continue uninterruptedly, and with perfect rhythm during ventricular contractions.

If it be assumed that the auricles are beating with a rhythm of their own, then a reconstruction of the cardiogram produced by auricular beats only would give the third line in Fig. 3. This curve was made by transferring to it those auricular cycles which were not obviously affected by ventricular contractions, for example k–k, and by fitting auricular cycles into the places deformed by ventricular contractions. This was done upon the assumption that the rhythm of the auricular beats remains the same as the rhythm which they have immediately before and after the corresponding ventricular systole.

Comparison of this reconstructed curve of auricular beats with the cardiogram obtained from the patient brings out the following significant facts: The contraction and relaxation of the ventricle in the first ventricular cycle (A, a–f) took place in the plateau stage of an auricular cycle—that is to say, they occurred at a time when the auricle produces no sharp waves on the cardiogram. We may therefore assume that the ventricular waves were but little affected by conditions arising in the auricles. The depression (g) in the line which follows the ventricular relaxation is deeper than most of the similar notches in other

symmetrical parts of the tracing. The same—indeed, more decided—depressions are seen in corresponding positions in ventricular cycles B, D, and G. All of these depressions correspond in point of time with depressions (auricular systoles and diastoles) on the reconstructed auricular cardiogram.

In the second ventricular cycle (B) notice should be taken of the fact that the ventricular contraction occurred exactly synchronously with an auricular systole and diastole, i. e., with a depression on the auricular cardiogram. The result is that the height of this ventricular wave is less than that of the preceding cycle. Its height is practically an algebraic summation of the positive wave produced by ventricular contraction, of which that recorded in the first ventricular cycle may be taken as a type, and of the negative wave produced by auricular activity. This state of affairs recurred in the seventh ventricular cycle (G); the effect upon the cardiogram was exactly the same.

In the third ventricular cycle (C) the positive wave (o) at the beginning of the ventricular plateau corresponds with an auricular systole in the reconstructed auricular cardiogram. The deep notch that follows this positive wave corresponds with an auricular depression. This combination of events was repeated in the eighth ventricular cycle (H).

The portion of the fourth ventricular cycle (D) corresponding with ventricular systole occupies a position immediately above an auricular plateau. Therefore this portion of the tracing escaped all deformation produced by the activities of the auricles. It should however be noted that the wave produced by ventricular contraction is higher than similar waves in other cycles. Perhaps the ascending limb of this wave was carried along to some extent by the ascending line (auricular diastole) on the reconstructed cardiogram. The two were exactly synchronous.

In order to bring out more clearly the effect of auricular beats on the form of the cardiogram, a curve of ventricular beats unaffected by auricular beats has been designed and placed exactly above similar points of the cardiogram obtained from the patient (line 1). It is not difficult to see that in almost every instance the original curve is obtained by algebraic addition of

the reconstructed auricular cardiogram to these assumed typical ventricular waves.

The Reconstructed Jugular Sphygmogram.—Facts having the same significance as those mentioned above are developed when a reconstructed curve of the jugular pulse (line 5) is compared with the curve (line 4) obtained from the patient. The reconstructed curve was made by transferring to it those portions of the original which were not obviously disturbed by ventricular contractions and by filling in the spaces left vacant, with waves similar to the above and timed with respect to the reconstructed auricular cardiogram. For the sake of clearness the pressure changes that occur during ventricular cycles have not been reproduced.

Examination of the portions of the record that correspond with the systole of the first ventricular cycle (A) shows that the first wave on the original was recorded synchronously with the first wave in the corresponding auricular cycle; that the second wave on the original corresponds with the second wave in the same auricular cycle in the reconstruction; and that the troughs between the waves have identical positions on both curves. A similar state of affairs recurred in cycles E and F.

In the part of the jugular pulse tracing which was obtained during ventricular systole of the second ventricular cycle (B), the first wave is immediately over an interauricular trough in the reconstruction. The former is considerably lower than the similar wave in the preceding cycle. On the descending limb of the second there is to be seen a break that was not found in the first cycle. This break in the descent of the wave was obviously caused by the wave immediately beneath it in the reconstructed curve. The same associations with similar effects are seen in cycle G.

Other instances of similar associations might be pointed out, but they become perfectly obvious when a comparison is made between the original and reconstructed venous tracings, and the assumed typical form of the venous waves that obtain during ventricular systole. The latter are represented in line 6 of the reconstruction. In almost every cycle a record of the original

form is obtained when the typified waves are added algebraically to synchronous waves in the reconstructed jugular record.

Discussion.—It is therefore obvious that the apex beat, jugular pulse, and brachial pulse of this patient show two perfectly regular rhythms. In the one rhythm, which is undoubtedly ventricular, the waves recur approximately every 2.12 sec.; in the other rhythm, which we have assumed to be auricular, the waves recur approximately every 0.6 sec. Furthermore it is obvious that these two rhythms are perfectly independent of one another. It is safe to affirm that the only known condition which can reproduce this state of affairs is that which is usually termed complete heart-block: it is the condition in which the impulse causing the auricles to contract does not reach the ventricles. These two regions of the heart therefore beat rhythmically and independently of one another.[8]

In subsequent parts of this paper abundant evidence will be developed which proves absolutely that the interpretation of these tracings is correct and that the disturbance in the rhythm of the heart is the result of a diminution of the conductivity at the auriculo-ventricular junction.

These tracings show some other features which are of no importance so far as concerns heart-block but which may be of sufficient interest to the physiologist to warrant a reference to them here.

The Auricular Sounds.—As has been observed in many other cases of heart-block in man, so here, the auricular beats produce audible but very feeble sounds. It was stated in the history of this patient that two sounds are associated with each auricular contraction. It seems probable that the first auricular sound, like part of the first sound produced by the ventricular contraction, is of muscular origin. This is of interest in view of the fact that evidence has been accumulating which seems to indicate that the first sound of the normally beating heart begins before ventricular contraction begins.[9] This observation has indeed been explained upon the assumption that the auricular con-

[8] References to the literature of this subject will be found in Part III.

[9] See Tigerstedt, *Ergeb. der Phys.*, 1902, i, pt. ii, 253.

traction plays a part in the production of the first heart sound. The findings in cases of heart-block certainly lend considerable support to this view. The second auricular sound of our patient is described by Dr. Thayer as being valvular in quality. In all probability it is produced either by the closure of, or by tension on, the auriculo-ventricular valves when, after having filled the ventricles, the auricles relax. These facts may be of some significance in connection with reduplications of the first heart sound.

The Origin of the Smaller Waves on the Arterial Sphygmogram.— In all of the brachial sphygmograms, but particularly in that of Fig. 2, small but distinct waves are scattered along the katacrotic limbs at more or less regular intervals. When the sphygmogram is compared with the cardiogram it is seen that these waves are synchronous with auricular systoles.[10] Similar waves have been seen by other investigators and have given rise to considerable confusion. They are undoubtedly of auricular origin, but the question arises: Are they transmitted through the arteries or the veins? The fact that they are produced by auricular beats would at first sight seem to favor the latter view. But positive evidence has been obtained that these waves are actually transmitted through the arteries. This evidence follows: On the rapidly revolving drum of the sphygmomanometer a continuous tracing was made of the pulsations transmitted to the instrument while the pressure which it exerted upon the arm was falling slowly, but constantly, from a point above the systolic pressure (150 mm. Hg.), to a point below the diastolic pressure (60 mm. Hg.). This tracing, which is reproduced in Fig. 5, shows that the amplitude of the small waves increases as the pressure on the arm approaches the diastolic arterial pressure (70 mm. Hg.), but that before the diastolic pressure is passed the amplitude of the small waves diminishes. According to Howell and Brush, the pressure on the arm at which a pulse-wave is reproduced with greatest amplitude is equal to the pressure at the trough of that wave. Therefore the pressure at the trough of the small waves

[10] Webster, *Glasgow Hosp. Reports*, 1900, iii, 413. MacKenzie, "Studies of the Pulse," etc., *ibid.*, 1902, p. 279.

exceeds the minimum arterial pressure by a small amount: it exceeds 70 mm. Hg. As this pressure is undoubtedly far in excess of that which obtains in the veins, the conclusion is obvious that the small waves seen on the sphygmogram are transmitted from the heart through the arteries.

Although this wave is undoubtedly produced by the contraction of the auricles, it is not necessary to assume that the contraction of the auricles actually projects blood into the arteries. Indeed, it is not likely that even under pathological conditions the auricles could contract with sufficient force to open the semilunar valves. Nor do we believe that the increase in the intraventricular pressure caused by the beats of the auricles would be sufficient to diminish the tension of the semilunar valves by enough to transmit a wave into the peripheral arteries. For when the auricles contract they send a wave into the relaxed ventricle. The ventricle, therefore, acts like a capacious reservoir and takes up all of the blood sent into it without allowing of any material rise of the intraventricular pressure. Another explanation, which has been suggested by F. Franck,[11] is that the contraction of the auricles transmits a shock to the root of the aorta by direct contact. In a case of the kind under consideration, in which the auricles have probably dilated and hypertrophied, such an explanation of the origin of the small waves seen in the arteries is not without plausibility.

Analysis of Tracings of the Jugular Pulse.—The tracings of the jugular pulse are of particular interest because the absence of ventricular beats in comparatively long sections of the tracing permits of a dissociation of those waves in the veins which are of auricular and of ventricular origin.

In most places two waves are recorded in each auricular cycle. The higher wave (k) corresponds in time with the auricular contraction, and the deep negative which follows it is probably synchronous with auricular relaxation. Following this, the pressure in the veins rapidly increases, reaches the second maximum (n), which is not so high as the first, from which it is separated by a second small negative wave. This second wave

[11] F. Franck, *Arch. de phys.*, 1890, 5th ser., ii, 395.

(n) is probably not the result of instrumental error, because there may be seen on the cardiogram a small but constant wave (n) which is synchronous with it. The two positive venous waves cannot be seen immediately after ventricular relaxation, but they become more and more distinct as the venous pressure increases in the intervals between ventricular beats.

So far as we are aware a wave similar to the smaller positive wave (n) has never been described before. Porter [12] does not refer to such a wave in his careful study of the intra-auricular pressure in the dog. Nor is it mentioned by Gerhardt [13] in connection with his studies of venous pulsations in man.

In the attempt to account for the occurrence of this smaller wave three possibilities present themselves for consideration:

(a) It is a filling wave. Following the relaxation of the auricle the blood continues to pour into this chamber and raises the pressure in it. But upon this assumption it is impossible to account for the depression which precedes the wave produced by auricular systole.

(b) It is a wave produced by contraction of the great veins. Under normal circumstances this cannot be observed, but it is possible that when the pressure in the great veins is high the effect of this contraction becomes evident.

(c) It is produced by active relaxation of the musculature of the ventricles. It has been observed by several investigators that during ventricular diastole the pressures in the ventricles and auricles may vary in opposite directions. In explanation of this phenomenon, v. Frey and Krehl [14] have offered the suggestion given above.

Although no evidence has been obtained which favors one or the other of the above mentioned possibilities, the fact remains that auricular systole may be preceded by a distinct positive wave in the veins of the neck. This fact should be borne in mind in connection with the interpretation of venous pulsations in general.

[12] Porter, *Jour. of Phys.*, 1892, xiii, 513.
[13] Gerhardt, loc. cit.
[14] v. Frey, and Krehl, *Arch. für Phys.*, 1890, 38.

DESCRIPTION OF TRACINGS ILLUSTRATING VARIOUS STAGES OF PARTIAL HEART-BLOCK.

It has been stated that some time after the admission of our patient into the hospital his condition began to improve. This improvement was indicated, among other ways, by a change in the condition of the heart. At first the complete heart-block rarely gave way to partial heart-block in which the rate of auricular contractions was to the rate of ventricular contractions as 4:1, or as 3:1. Later, this degree of partial block became more or less constant, so that it was difficult to make observations at times when the block was complete. Still later, the rhythm was usually 2:1; and just before the condition of the heart became perfectly normal there was a stage during which a ventricular contraction failed to follow an auricular contraction only occasionally.

An example of a 4:1 rhythm is reproduced in Fig. 6. In this curve it may be seen that there are four auricular contractions for one ventricular contraction, and that the fourth auricular contraction is immediately followed by the ventricular contraction. This may have been a coincidence; but positive evidence has been obtained that such was not the case. This evidence will be given in connection with the special experiment during the course of which this tracing was obtained.

Figs. 7 and 8 (1st part) are examples of a 3:1 rhythm. There are three auricular beats, the last of which is immediately followed by the ventricular beat. Here again evidence has been obtained that this relation of ventricular to auricular contractions was not the result of chance. For, in the experiment of which Fig. 8 forms a part, when by means of a certain procedure the heart-rate was increased, there was a proportional increase of auricular and ventricular rates, and the relation of the ventricular to the third auricular contraction remained unaltered. Fig. 9 shows one cycle of a 4:1 rhythm in the midst of cycles of a 3:1 rhythm.

In Fig. 10 is reproduced an instance of a 2:1 rhythm. And finally the normal sequence of the heart-beat is shown in Fig. 11.

In Table I are given the rates of auricular and ventricular

contractions which obtained at the time the tracings used for the illustrations mentioned above were made.

TABLE I.

AURICULAR AND VENTRICULAR RATES IN FIGURES USED TO ILLUSTRATE THE VARIOUS STAGES OF HEART-BLOCK.

Fig.	Vent. Rate per Min.	Aur. Rate per Min.
Fig. 1 complete block.	27.7	101.5
Fig. 6, 4:1	24.0	96.0
Fig. 7, 3:1	24.8	74.4
Fig. 10, 2:1	30.5	61.0
Fig. 11, normal.	57.7	57.7

No systematic study has been made of the variations of the rate of the conduction of the impulse from auricles to ventricles in the different stages of block. But in all of our tracings it may be seen that the interval between the beginning of auricular systole and of ventricular systole—the intersystolic period—is longer than under normal conditions. In Fig. 8 a tracing is reproduced which shows the lengthening of this period associated with the transition from a partial block with a 3:1 rhythm, to complete block.[15]

THE EFFECT OF EXTRINSIC INFLUENCES ON THE RATES OF THE AURICLES AND VENTRICLES IN COMPLETE AND PARTIAL HEART-BLOCK.

Introduction.—It has been demonstrated that when our patient first came under observation he was suffering from complete heart-block. As in complete heart-block the auricles and ventricles beat independently of one another, the opportunity was afforded of studying, in man, the independent reactions of auricles and ventricles to extrinsic stimuli,—stimuli carried to the heart through the medium of its extrinsic nerves or through the medium of the blood. Observations of this kind should have a peculiar value in that they may be made under conditions which

[15] Wenckebach (*Die Arhythmie*, etc., Leipzig, 1903) first called attention to this phenomenon in man.

cannot be obtained in animal experimentation. For example, it is impossible to produce complete heart-block in animals without opening the thorax, and the studies can be made only in anæsthetized animals. And furthermore, at the time this work was undertaken no satisfactory means of producing complete heart-block in warm-blooded animals were known. It could with certainty be produced only by destroying completely the connection between auricles and ventricles.

Many observers have noted that in cases of Stokes-Adams disease the arterial pulse-rate is not materially affected by conditions which, under normal circumstances, cause a change in the pulse-rate.[16] The significance of this observation was not recognized because, in the first place, the investigators, as a rule, did not know that they were dealing with heart-block, and in the second place because they did not determine the rate of auricular contractions.

We have studied the effect of many conditions upon the rates of the auricles and ventricles in complete and partial block. The method employed was to record the cardiac impulse in the usual way immediately above a chronograph marking fifths of seconds. The rate of auricular and ventricular contractions was then determined by counting out the waves on the fixed records. The rates determined in this way are accurate to about the tenth of a second.

THE EFFECT OF POSTURAL CHANGES.

It was first shown by Mosso that blood accumulates in the dependent portions of the body when its position is changed from the recumbent to the erect,[17] and it has since been shown that the circulatory changes which are usually associated with this change in position may be obviated by compressing the lower portions of the body.[18] It is therefore probable that the increase in heart-rate which always accompanies the assumption of the

[16] References to some of these observations will be found in an article by Jaquet, *Deutsches Arch. für klin. Med.*, 1902, lxxii, 77.

[17] Mosso, *Arch. ital. de biol.*, 1884, v, 130.

[18] Erlanger and Hooker, *Johns Hopkins Hosp. Reports*, 1904, xii, 145.

erect posture, is caused by the same factor that induces the increase in heart-rate during hæmorrhage. It has been shown that in all probability the heart-rate is increased by hæmorrhage through a diminution in the tone of the vagus centre.[19] It may therefore be presumed that alterations of the heart-rate with changes in posture are vagus effects.

Complete Block.—The results obtained in a few typical experiments on our subject testing the effect of posture are collected in Table II. These results demonstrate that with the change from the recumbent to the erect posture the ventricular rate is not materially affected, whereas the auricular rate shows the acceleration which normally affects the whole heart.

TABLE II.

EFFECT OF POSTURE.

Date.	Posture.	No. of Vs.	Duration in $\frac{1}{5}$ Sec.	V. Rate per Min.	No. of As.	Duration in $\frac{1}{5}$ Sec.	A. Rate per Min.
i, 11, '05	Lying	15	179	24.96	61	179	102.2
	Standing	15	180	24	69	190	109
i, 11, '05	Lying	16	194	24.7	64	193	99.5
	Standing	17	205	24.9	73	208	105.3
xii, 22, '04	Lying	1	13.7	21.8	1	3.86	77.7
	Standing	1	13.4	22.4	1	3.2	93.2
xii, 22, '04	Lying	5	70	21.4	18	70	77.2
	Standing	3	40	22.5	12	39	92.3
xii, 22, '04	Lying	1	11	26.7	3:1	—	80.1
	Standing	1	10.3	29.1	3:1	—	87.3

Partial Block.—The objection might be raised that at the time these experiments were performed the ventricular muscle, owing to some pathological state, was not capable of responding to influences arriving in it. This objection is met in the following experiment, and in many other experiments to be mentioned later. The first two experiments dated December 22 were performed, as the table indicates, on the same day, but there was a considerable

[19] The literature on this subject has been collected by Fredericq, *Acad. roy. d. méd. à Belgique*, 1884–5, viii, 26.

interval of time between them. Shortly after the second experiment had been completed another similar experiment was performed (the last dated Dec. 22). But to our great surprise it was found that the complete block had given way to a perfectly typical partial block with a 3 : 1 rhythm. This was the first evidence obtained of an improvement in the patient's condition. Now, as may be seen in the table, when the erect posture was assumed the rate of the auricles was increased, not so markedly, however, as in the earlier experiment, and this increase was associated with a proportional increase in the rate of the ventricles. This experiment proves at one and the same time that we have to do here with a 3 : 1 rhythm, and that the ventricular muscle is capable of beating faster than it does when the block is complete. It is only necessary that impulses arrive in it.

THE EFFECT OF MUSCULAR EXERCISE.

Hering states [20] that the acceleration of the heart-rate which is associated with muscular exertion does not occur in rabbits when the accelerators are cut. It is therefore evident that at least some of the cardiac acceleration which is associated with muscular exertion results from the action of the accelerator nerves upon the heart.

We have tested the effect on the rates of the auricles and ventricles of slight and severe muscular exercise. Both forms of exercise were taken with the patient in the recumbent posture.

Moderate Exercise.

The light exercise consisted of gentle flexion and extension of the legs at the knee-joints, and of clenching and unclenching the hands. This form of exercise was chosen because it did not jar the recording apparatus and consequently it was possible to make tracings while the patient was exercising.

Complete Block.—The results obtained are given in Table III. In this table it may be seen that light exercise has practically no effect upon the rate of the ventricles but that the auricular rate, as a rule, shows the usual acceleration.

[20] Hering, *Zent. für Phys.*, 1894, viii, 75.

TABLE III.

EFFECT OF MODERATE EXERCISE.

Date.		No. of Vs.	Duration in $\frac{1}{5}$ Sec.	V. Rate per Min.	No. of As.	Duration in $\frac{1}{5}$ Sec.	A. Rate per Min.
ii, 4, '05	Before	9	115.5	23.4	31	112.2	82.9
	During	12	151.4	23.8	40	149.2	80.4
	After	12	163	22.1	45	155.8	86.6
ii, 4, '05	Before	11	142.4	23.2	38	133	85.7
	During	14	186.8	22.5	56	187.4	89.6
	After	10	128.6	23.3	39	128.5	91
ii, 4, '05	Before	10	127.4	23.5	34	129.4	78.8
	During	14	184.5	22.8	50	173.8	86.3
	After	10	126.6	23.7	38	126	90.5
ii, 4, '05	Before	11	147.4	22.4	39	147	79.6
	During	13	166.6	23.4	45	164	82.3
	After	11	145	22.8	42	145.6	86.5
ii, 7, '05	Before	11	134.2	24.6	33	132.1	74.9
	During	8	97.2	24.7	25 (?)	99.6	75.3 (?)
	"	7	85.3	24.6	22	82.7	79.8
	After	11	133.3	24.8	36	134	80.6
ii, 7, '05	Before	11	133.2	24.8	33	132.6	74.6
	During	8	97	24.7	23	90	76.7
	"	8	97.2	24.7	25	95.4	78.6
	After	8	97.2	24.7	25	92.2	81.4
ii. 7, '05	Before	12	146.4	24.6	38	145.2	78.5
	During	7	85	24.7	20	77.8	77.1
	"	8	97.7	24.6	26	97.7	79.9
	After	9	110	24.7	27	104	77.9
ii, 7, '05	Before	15	182	24.7	44	176	75
	During	9	109.8	24.6	25	98.4	76.2
	"	9	108.8	24.8	30 (?)	109	82.6 (?)
	After	4	48	25	11	39.8	82.9
	"	8	97.6	24.6	27	99	81.8
	"	9	110.2	24.5	26	101	77.2
	During	8	97.6	24.6	27 (?)	105	77.2 (?)
	"	9	110	24.6	31	111	83.8
	After	3	37	24.3	11	40.2	82
ii, 1, '05	Before	6	69.2	26	3:1	—	78
	During	17	193	26.4	3:1	—	79.2
	After	14	156.6	26.8	3:1	—	80.4
ii, 1, '05	Before	10	117.6	25.5	3:1	—	76.5
	During	15	172.8	26	3:1	—	78
	After	15	168.2	26.8	3:1	—	80.4
ii, 1, '05	Before (Irregular)	6	71	25.3	3:1	—	76
	During	11	124	26.6	3:1	—	79.8
	"	9	100.3	26.9	3:1	—	80.7
	After	12	136.6	26.4	3:1	—	79.2

Partial Block.—During partial block the slight increase in the rate of the auricles produced by exercise is naturally associated with a proportional increase in the rate of the ventricles.

Severe Exercise.

The severe exercise consisted of repeated vigorous elevation and depression of the arms. Tracings could not be made while the exercise was being taken, but the record was begun at the moment the patient came to rest.

Complete Block.—The results given in Table IV demonstrate that vigorous muscular exercise increases the rate of the ventricles as well as that of the auricles. The condensed table does not convey the proper conception of the degree of ventricular acceleration; for after the cessation of exercise, the ventricular rate rapidly returns to that which had previously existed. This is illustrated in Table V, in which are given the details of one of the experiments of Table IV.

Partial Block.—One experiment was performed while the block was partial, with a 2 : 1 rhythm (Jan. 12; Table IV). Examination of the column in which is given the duration of the auricular beats will show that the auricular rate suffered the usual acceleration. But in the column giving the duration of the ventricular cycles it may be seen that exercise decreased the ventricular rate. It may also be seen that while the auricular rate was decreasing subsequent to the exertion, a place was reached at which the ventricular rate increased abruptly. These apparently anomalous changes in the ventricular rate were caused by changes in the completeness of the block. Thus before taking the exercise the rhythm was 2 : 1, immediately after it was 3 : 1, and later it again became 2 : 1.

THE EFFECT OF INHALATIONS OF AMMONIA.

It has been definitely determined that in the rabbit the inhalation of ammonia may slow the heart-rate through reflex action on the cardio-inhibitory centre.[21] Therefore several experiments were performed to test the effect of ammonia on the rates of

[21] F. Franck, *Travaux du laboratoire de Marey*, 1876, ii, 221.

TABLE IV.

EFFECT OF SEVERE EXERCISE.

Date.		No. of Vs.	Duration in $\frac{1}{5}$ Sec.	V. Rate per Min.	No. of As.	Duration in $\frac{1}{5}$ Sec.	A. Rate per Min.
i, 2, '05	Before	9	117	23.1	33	110	90
	After	8	95	25.3	30	82	95
i, 2, '05	Before	11	133	24.8	37	127	87.4
	After	10	119	25.2	37	110	100.9
i, 7, '05	Before	10	131	22.9	41	143	86
	After	10	124	24.2	42	127	99.2
i, 7, '05	Before	9	114	23.7	29	102	85.3
	After	12	133	27.1	40	123	97.6
			Partial Block				
i, 12, '05	Before	21	7.6 (Av.)		2:1	3.8	
	After	1st	8.6		3:1	2.5	
	"	6th	9.2		3:1	3.06	
	"	11th	9.4		3:1	3.13	
	"	16th	9.6		3:1	3.2	
	"	23d	9.6+		3:1	3.2	
	"	24th	6.5		2:1	3.25	
	"	31st	7.2		2:1	3.6	
	"	36th	7.4		2:1	3.7	
	"	41st	7.3		2:1	3.65	
	"	46th	7.4		2:1	3.7	
	"	50th	7.5		2:1	3.75	

TABLE V.

EFFECT OF SEVERE EXERCISE (JAN. 2, 1905).

Before Exercise.			After Exercise.		
No. of Vs.	Duration in $\frac{1}{5}$ Sec.	Duration of As. in $\frac{1}{5}$ Sec.	No. of Vs.	Duration in $\frac{1}{5}$ Sec.	Duration of As. in $\frac{1}{5}$ Sec.
1	13.6	3.4 — 3.2	1	11.4	3 — 3
2	13.4	3.4 — 3.2	2	11.5	3 — 3
3	13.4	3.3 — 3.2	3	11.5	3 — 3
4	13.2	3.4 — 3.2	4	11.6	3+
5	12.8	3.3 — 3.2	5	12	3+
6	13	3.4 — 3.2	6	12	3+
7	12.6	3.4 — 3.2	7	12.2	3
8	12.2	3.5 —	8	12.2	3
9	12	3.2 —			

auricles and ventricles of our patient. The patient was given a bottle containing a dilute solution of ammonia from which to inhale. He was asked to take as much as he could bear, but it is impossible to tell how well he performed his part of the experiment. The results obtained were neither striking nor concordant, and no conclusions can be drawn from them. The results of two experiments which have been carefully calculated are given below (Table VI).

TABLE VI.

EFFECT OF INHALING AMMONIA.

Date.		No. of Vs.	Duration in $\frac{1}{5}$ Sec.	V. Rate per Min.	No. of As.	Duration in $\frac{1}{5}$ Sec.	A. Rate per Min.
i, 2, '05	Before	4	44.4	27	15	46	97.8
	During	5	57.4	26.1	19	58.4	97.4
i, 2, '05	Before	5	56.8	26.4	19	58.2	97.9
	During	5	57.2	26.2	18	55.4	97.5
	After	7	79	26.6	27	80.6	100.6

THE EFFECT OF INHALATIONS OF OXYGEN.

The statement is made by Hoffmann that in cases of Stokes-Adams disease the pulse-rate may be increased by inhalations of oxygen.[22] In the case of our patient inhalations of oxygen have proved to be absolutely without effect. The results of a prolonged experiment are given below (Table VII).

TABLE VII.

EFFECT OF INHALING OXYGEN.

	No. of Vs.	Duration in $\frac{1}{5}$ Sec.	V. Rate per Min.	No. of As.	Duration in $\frac{1}{5}$ Sec.	A. Rate per Min.
Before	7	79.5	26.4	25	80.5	93.5
During	5	57.6	26.1	18	57.5	94.4
"	4	46	26.1	15	46.8	96.2
"	7	82.2	25.5	26	82.6	94.5
"	6	70	25.7	22	70.6	93.6
"	6	69.2	26	22	70.4	93.6
After	6	69.6	25.9	22	68.4	96.4
"	7	79.6	26.4	25	81	92.6

[22] Hoffmann, *Zeit. für klin. Med.*, 1900, xli, 357.

THE EFFECT OF INSPIRATORY AND EXPIRATORY TETANUS.

Inspiratory and expiratory tetanus seemed to increase the rates of auricles and ventricles in complete block. But the effect was not marked, probably because the experiments could not be continued long enough to yield conclusive results.

THE EFFECT OF ASPHYXIA.

It is stated that in all stages of asphyxia the heart-rate is slowed through increased activity of the cardio-inhibitory centre.[23] To test the effect of asphyxia we had the patient hold his breath as long as he could.

Complete Block.—The results obtained in many experiments are given below (Table VIII). It may be seen that while the breath is being held the rates of the auricles and ventricles are, as a rule, not materially affected. Perhaps toward the end of this period there is a tendency for the auricular rate to increase and for the ventricular rate to decrease. During the period of dyspnœa which comes on after the breath can no longer be held, this effect becomes much more striking: there is now a decided increase in the rate of the auricles and a definite decrease in the rate of the ventricles. We have many similar experiments but those already given suffice to show that the slowing of the ventricular rate seen here is in all probability not a vagus effect. For if the rate of the ventricles is slowed by the action of asphyxia on the cardio-inhibitory centre, then the rate of the auricles should be affected in the same direction. The significance of the slowing of the rate of the ventricles by asphyxia will be discussed later, in connection with other more conclusive experiments.

Partial Block.—When the block is partial, the ventricular rate varies directly as the auricular rate provided the degree of block does not change. But frequently the increase of the auricular rate is associated with a change from partial block with a 3:1 rhythm to complete block. The ventricular rate is then distinctly, sometimes markedly, slowed. The phenomenon is similar to that which was seen to follow muscular exertion.

[23] Starling, *Schafer's Text-book of Physiology*, 1900, ii, 387.

TABLE VIII.

EFFECT OF HOLDING BREATH.

Date.		No. of Vs.	Duration in $\frac{1}{5}$ Sec.	V. Rate per Min.	No. of As.	Duration in $\frac{1}{5}$ Sec.	A. Rate per Min.	Remarks.
ii, 15, '05	Before	5	59	25.4	16	59.4	80.8	
	"	5	58.6	25.6	15	56.2	80.1	
	During	4	46.6	25.7	12	46.1	78.1	
	"	3	35.4	25.4	10	39.0	76.9	
	"	3	36.3	24.8	9	35.7	75.7	
	After	4	49.8	24.1	15	50.3	89.0	Preceded by long illegible interval.
	"	4	47.9	25.0	14	47.4	88.5	
ii, 15, '05	Before	6	70.3	25.6	19	71.6	79.6	
	"	6	70.2	25.6	18	69.5	77.6	
	During	3	35	25.7	10	37.0	81	
	"	3	35	25.7	9	35.0	77.2	
	"	3	36.2	24.9	9	34.2	79	
	After	3	35.8	25.1	11	37.1	88.9	Preceded by an illegible interval.
	"	3	34.9	25.8	10	34.5	86.9	
ii, 15, '05	Before	6	69.7	25.8	19	71.7	79.5	
	"	5	58.1	25.8	15	56.0	80.3	
	During	3	34.9	25.8	10	36.7	82.6	
	"	3	35.2	25.6	9	34.3	78.7	
	"	3	36.1	24.9	10	37.0	81.1	
	After	3	37.6	23.9	10	35.8	83.8	
	"	4	50.2	23.9	14	50.7	82.9	
	"	4	49.0	24.5	15	50.2	89.6	
ii, 15, '05	Before	5	58.3	25.7	15	57.3	78.6	
	"	6	69.2	26.0	18	69.8	77.3	
	During	4	46.7	25.7	12	48.0	75.0	
	"	4	47.3	25.4	12	46.4	73.7	
	"	3	36.2	24.9	9	34.6	78.0	
	After	3	37.0	24.3	10	36.5	82.2	
	"	4	50.0	24	15	51.5	87.4	
	"	4	47.6	25.2	14	48.9	85.8	
	"	5	56.5	26.5	15	57	79.0	
ii, 9, '05	Before	10	100	30	3 : 1	—	90	
	During	7	71	29.6	3 : 1	—	88.8	
	"	5	52	28.8	3 : 1	—	86.4	
	After	6	57.6	31.3	3 : 1	—	93.9	Preceded by an illegible interval.
	"	8	78.8	30.3	3 : 1	—	91.5	
	"	7	70.6	29.8	3 : 1	—	89.4	
ii, 9, '05	Before	9	93.2	29	3 : 1	—	87	
	During	4	41.5	28.9	3 : 1	—	86.7	
	"	3	31.8	28.3	3 : 1	—	84.9	
	"	3	31.2	28.8	3 : 1	—	86.4	
	After	3	36.8	24.5	4 : 1 (?)	—	(?)	Preceded by an illegible interval.
	"	5	49.6	30.2	3 : 1	—	90.7	
	"	7	72.2	29.1	3 : 1	—	87.3	

TABLE VIII.—*Continued.*

Date.		No. of Vs.	Duration in $\frac{1}{5}$ Sec.	V. Rate per Min.	No. of As.	Duration in $\frac{1}{5}$ Sec.	A. Rate per Min.	Remarks.
ii, 9, '05	Before	9	92.8	29.1	3 : 1	—	87.3	
	During	4	42.5	28.2	3 : 1	—	84.6	
	"	3	32.0	28.1	3 : 1	—	84.3	
	"	3	31.4	28.7	3 : 1	—	86.1	
	"	1	14.5	20.7(?)	7	22.8	92.1	Followed by illegible interval: apparently ventricular stoppage.
	After	4	39.2	30.6	3 : 1 (?)	—	91.8(?)	
	"	3	40.4	22.3	14	45.2	92.9	
	"	4	50.8	23.6	14	45.2	92.9	Interval of alternating 3 : 1 and 4 : 1 rhythms.
	"	4	41.0	29.3	3 : 1	—	87.9	
	"	4	40.7	29.5	3 : 1	—	88.5	
	"	4	41.8	28.7	3 : 1	—	86.1	
ii, 9, '05	Before	8	83	28.9	3 : 1	—	86.7	
	During	4	43.8	27.4	3 : 1	—	82.2	
	"	3	32.6	27.6	3 : 1	—	82.8	
	"	3	31.7	28.4	3 : 1	—	85.2	
	"	1	14.3	21.0	4	13.2	90.9	Followed by illegible interval: apparently ventricular stoppage.
	After	1st	15.2	19.7	4	12.5	96	
	"	2d	11.0	27.3	4	12.5	96	
	"	3d	13.8	21.7	4	12.8	93.6	
	"	4th	12	24.9	4	13.0	92.3	
xii, 22, '04	During	1st	10.8	—	3.5	—	—	3 : 1
	"	2d	11	—	3.5	—	—	3 : 1
	"	3d	10.8	—	3.5	—	—	3 : 1
	"	4th	11	—	3.5	—	—	3 : 1
	"	5th	11	—	3.6	—	—	3 : 1
	"	6th	11	—	3.6	—	—	3 : 1
	"	7th	11	—	3.5	—	—	3 : 1
	"	8th	10.8	—	3.5	—	—	3 : 1
	"	9th	10.8	—	3.4	—	—	3 : 1
	"	10th	10.8	—	3.5	—	—	3 : 1
	"	11th	10.6	—	3.4	—	—	3 : 1
	"	12th	10.6	—	3.4	—	—	3 : 1
	"	13th	10.6	—	3.4	—	—	3 : 1
	"	14th	10.6	—	3.4	—	—	3 : 1 As–Vs interval lengthening.
	"	15th	10.6	—	3.4	—	—	3 : 1 As–Vs interval lengthening.
	"	16th	13.5	—	3.3 — 3.3	—	—	Complete block.
	"	17th	13.5	—	3.2 — 3.2	—	—	Complete block.

THE EFFECT OF ATROPINE.

Dehio first suggested the use of atropine for the purpose of determining the part played by the inhibitory apparatus in the causation of the slow or irregular heart-beat. This method of diagnosis has been used by him in a case of Stokes-Adams disease, with the result that the administration of atropine had no effect upon the pulse-rate.[24] This observation, together with others that have been made since, indicates that in cases of Stokes-Adams disease the vagi do not act upon the ventricles. But no one has compared the effect of atropine on the rate of the ventricles with its effect on the rate of the auricles in cases of this kind.

We have performed three experiments, two to test the effect of atropine while the heart-block was complete, and one while the heart-block was partial. The experiments were performed as follows: Several determinations of the rates of the auricles and ventricles were made before giving the atropine. Then, after giving one milligram of atropine subcutaneously, estimations of the rates were made every few minutes for several hours, and then at rather infrequent intervals for even a longer period of time. The patient remained in bed throughout the course of the experiment.

The Effect of Atropine in Complete Block.—The first experiment was performed at a time when the subject was having frequent syncopal attacks (Table IX). At the beginning of the experiment these recurred so frequently and irregularly that an accurate determination of the normal ventricular rate was impossible. We shall therefore simply refer to the fact that after a preliminary slowing, following the injection of atropine, the rate of auricular contractions was decidedly increased. After the administration of the atropine the rate of ventricular contractions also increased, but the ventricles resumed the rate which they had had at the opening of the experiment before the auricular rate had begun to decrease.

The second experiment was performed under more favorable

[24] Dehio, *St. Petersburger med. Woch.*, 1892, xvii, 1.

circumstances. The heart-rate was perfectly regular and it was therefore an easy matter to determine the exact action of the atropine on the auricles and ventricles.

TABLE IX.

EFFECT OF ATROPINE (JANUARY 16, 1905).

Minutes after Injection of Atropine.	No. of Vs.	Duration in $\frac{1}{5}$ Sec.	V. Rate per Min.	No. of As.	Duration in $\frac{1}{5}$ Sec.	A. Rate per Min.	Remarks.
Before.	26	318	24.5	108	318	101.9	1 mgr. atropine subcut.; slight attack.
2	12	138	26.1	50	148	101.3	
4	8	105	22.9	37	109	101.8	
5	9	101	26.7	34	105	97.2	
8	11	117	28.2	45	128	105.5	
11	12	128	28.1	47	132	106.8	
15	15	160	27.8	59	160	110.6	
20	14	151	27.8	56	151	110.2	
25	13	143	27.3	52	138	113	
30	12	133	27.1	49	131	112.2	
40	13	144	27.1	52	137	113.9	
50	13	147	26.5	60	160	112.5	
60	10	112	26.8	42	112	112.5	
70	14	161.5	26	61	162.5	112.6	
80	10	116	25.9	44	117	112.8	
90	13	152.5	25.6	57	154	111.1	
100	13	153	25.5	57	155	110.3	
110	10	115.5	26	43	118	109.3	
120	12	139	25.9	57	152	112.5	
150	35	410	25.6	147	410	107.6	

Inspection of Table X will show that the rate of ventricular contractions was practically uninfluenced by the atropine, excepting a very slight reduction of the ventricular rate throughout the course of the experiment. The auricles responded to the atropine as the whole heart normally responds: after a preliminary slowing, the rate began to increase and reached a maximum in about forty-four minutes. The auricular rate was increased from 67 to 95.1 beats per minute. It then decreased slowly and somewhat irregularly. The conclusion warranted by this experiment is perfectly evident: when the block is complete atropine has no effect upon the ventricular rate, but it has the usual accelerating effect upon the auricular rate.

TABLE X.

EFFECT OF ATROPINE (FEBRUARY 17, 1905).

Time.	No. of Vs.	Duration in $\frac{1}{5}$ Sec.	V. Rate per Min.	No. of As.	Duration in $\frac{1}{5}$ Sec.	A. Rate per Min.	Remarks.
11:00	9	108	25	24	108.6	66.3	
11:05	11	131	25.2	29	129	67.4	
11:10	13	154.6	25.2	34	153.2	66.5	
11:11							1 mgr. atropine subcut.
11:13	10	119.2	25.2	27	119.8	67.6	
11:15	11	130.8	25.2	29	132	65.9	
11:17	12	141.5	25.4	29	140.6	61.9	
11:20	10	117.3	25.6	24	118.4	60.8	
11:23	11	129.7	25.4	33	143.3	69.1	
11:26	10	118.8	25.2	29	119.7	72.7	
11:29	9	106.6	25.3	28	106.1	79.2	
11:31	11	130.6	25.3	37	131	84.7	
11:34	11	128.7	25.6	37	128.8	86.2	
11:37	10	119	25.2	35	118	89	
11:40	11	131	25.2	40	130.2	92.2	
11:45	11	131.9	25	40	131.6	91.2	
11:50	11	131.5	25.1	41	131.8	93.3	
11:55	12	144	25	46	144.3	95.1	Throat dry.
12:00	11	133.5	24.7	42	133	94.7	
12:05	11	132	25	41	131	93.9	
12:10	11	131.4	25.1	40	130.8	91.7	
12:15	10	122	24.6	38	121.5	93.8	
12:20	11	135.8	24.3	41	136	90.4	
12:25	11	136.5	24.2	40	134.2	89.2	
12:30	10	124.5	24	38	126.2	90.3	
12:35	10	124.8	24	37	124.1	89.4	
12:40	9	111.7	24.2	34	114.2	89.3	
12:45	11	136.3	24.2	39	135	86.7	
12:50	11	136.3	24.2	39	135.4	86.4	
12:55	9	111.6	24.2	32	112.5	85.3	
1:00	11	136.8	24.1	41	135.9	90.5	
1:05	10	124.3	24.1	35	122.3	85.9	
1:10	9	111.1	24.3	33	110	90	
1:15	11	136.2	24.2	38	136	83.8	
1:20	10	123.1	24.4	36	123.8	87.2	
1:25	10	124.3	24.1	35	124	84.7	
1:30	11	137.2	24.1	39	139.2	84	
2:14	10	125	24	36	125	86.4	Lunch.
3:13	11	133.4	24.7	37	135	82.2	
3:58	11	131	25.2	32	130.6	73.5	
7:05	11	135	24.4	34	135.8	75.1	Supper.

The Effect of Atropine in Partial Block.—The effect of the administration of atropine while the block is partial is shown in Table XI. In this table it may be seen that the effect upon the auricles was the same as in the preceding experiment. After a preliminary slowing, the auricular rate increases rather rapidly

and reaches a maximum in forty minutes. The rate was increased from 80 to 102.3 beats per minute. It then decreased slowly and irregularly.

The ventricular rate varied irregularly, but in almost every instance these irregularities find a simple explanation. At the beginning of the experiment the rhythm was 3:1. Therefore the frequency of the ventricular beats was just one third of that of the auricular beats. And with the increase of the auricular rate there was a proportional increase of the ventricular rate, because the ventricular rate is determined by the auricles. At 10:10 A.M. the ventricular rate suddenly decreased from 29.3 to 23.8 beats per minute. It will be seen that at this moment the rhythm became 4:1. In the two determinations which followed the block became complete. Here the variations of the ventricular rate bear no relation to the variations of the auricular rate. In the next two determinations the rhythm again became 4:1 and consequently the ventricular rate follows the auricular rate. But after this determination, and practically until 11:40 A.M. the block was complete: the ventricular rate was slow and its slight variations bore no relation to those of the auricles. Then there was some fluctuation between a 3:1 rhythm and complete block, but at 12:30 P.M. the rhythm became 3:1, and remained so until 2:15 P.M., when it became 2:1. This change, as might be expected, was associated with a marked increase in the rate of the ventricles.

In this experiment it is again clear that the atropine has had no direct effect upon the ventricles. In many places the ventricular rate has been increased but in each one of these places it can be shown that the increase in rate has been effected indirectly through the influence of the auricles upon the ventricles.

INFLUENCES WHICH AFFECT THE DEGREE OF PARTIAL BLOCK.

It has been shown that the degree of partial block may be altered by exercise, by asphyxia, and by atropine. It will be recalled that, apparently, the block is increased only when the auricular rate increases. We are therefore inclined to believe that the increase in the auricular rate is the important factor

TABLE XI.

EFFECT OF ATROPINE (FEBRUARY 10, 1905).

Time.	No. of Vs.	Duration in $\frac{1}{5}$ Sec.	V. Rate per Min.	No. of As.	Duration in $\frac{1}{5}$ Sec.	A. Rate per Min.	Remarks.
9:40	12	134.2	26.8	3:1		80.4	
9:45	6	67.6	26.6	3:1		79.8	
9:50	11	122.3	27	3:1		81	
9:52							1 mgr. atropine subcut.
9:53	15	156.6	28.7	3:1		86.1	
9:55	8	90.4	26.5	3:1		79.5	
9:57	13	146.2	26.7	3:1		80.1	
10:00	11	124.3	26.5	3:1		79.5	
10:03	13	138.2	28.2	3:1		84.6	
10:06	16	164	29.3	3:1		87.9	
10:10	9	113.2	23.8	4:1		95.2	
10:12	13	155.8	25	50	152.6	98.3	
10:14	13	154.5	25.2	50	155	96.9	
10:16	11	137.3	24	4:1		96	
10:18	14	168	25	4:1		100	
10:21	12	143	25.2	48	141	102.1	
10:24	10	116.6	25.7	39	116	100.9	
10:28	15	180	25	61	179.6	101.9	
10:32	11	132.6	24.9	45	132	102.3	
10:36	12	144.2	25	48	144.6	99.6	
10:40	15	178.3	25.2	59	178.6	99.1	
10:45	10	121	24.8	40	122	98.3	
10:50	14	146.8	28.6	47	153.6	91.7	
10:55	14	156.2	26.9	50	163.2	91.9	
11:00	12	135.3	26.6	40	129	93	Throat dry.
11:05	11	125.3	26.3	37	123.3	90	
11:10	12	124.2	29	3:1		87	
11:15	15	163.8	27.5	46	157.7	87.5	All but last Vs. = 3:1 rhythm.
11:20	13	152.2	25.6	42	143.2	88	
11:25	11	131	25.2	37	123	90.2	
11:30	13	147	26.5	39	139.8	83.6	Asleep.
11:35	12	142	25.3	40	134	82.8	Asleep.
11:40	11	132	25	37	123.3	90	Asleep.
11:45	12	131.8	27.3	3:1		81.9	
11:50	11	123.5	26.7	33	115.5	85.2	All but 1st 2 Vs. = 3:1.
12:00	12	135.4	26.6	3:1		79.8	
12:05	14	158.7	26.5	3:1		79.5	
12:10	11	123	26.8	3:1		80.4	
12:15	14	156.1	26.9	43	149.8	86.1	1st $\frac{1}{2}$ complete block: then 3:1.
12:20	12	145.3	24.8	42	138.3	91.1	
12:25	13	146.2	26.7(?)	40	139	86.3	Pulse irregular.
12:30	15	166.3	27.1	3:1		81.3	
12:35	12	132.8	27.1	3:1		81.3	
12:40	12	129.2	27.9	3:1		83.7	
12:45	15	162.8	27.6	3:1		82.8	Asleep.
12:50	11	121.6	27.1	3:1		81.3	
12:55	11	122.2	27	3:1		81	
1:00	11	124	26.6	3:1		79.8	Lunch.
2:15	15	147.5	30.5	2:1		61	
3:20	10	94.8	31.6	2:1		63.2	
6:05	12	144.6	24.8	3:1		74.4	Supper.

here, just as we believe it to be the important factor in causing the syncopal attacks.

The reduction in the conductivity at the auriculo-ventricular junction which is seen in these experiments and which is associated with an increase in the auricular rate, is a phenomenon that is frequently seen in the cold-blooded heart. If, for example, the rate of the excised heart of the terrapin be very markedly increased by exposing it to an elevated temperature, a 2 : 1 rhythm may develop.[25] In connection with an experiment of the same kind Gaskell states that "anything which diminishes the conductivity of any part of the tissue will tend to cause a block to the contraction wave at that part; and the question whether a block will be manifested or not will depend upon the rate of the heart-beat in relation to the extent of the diminution in the conductivity in the damaged part." [26]

THE SYNCOPAL ATTACKS.

During the course of these investigations we have had many opportunities of studying the behavior of the heart during the syncopal attacks which form one of the characteristic features of Stokes-Adams disease. The subjective symptoms of these attacks were usually very mild; in them the patient never actually lost consciousness, although he undoubtedly would have fallen had he been up and about. The patient called them "dizzy spells."

The Behavior of the Heart during a Syncopal Attack is illustrated in Figs. 12, 13, and 14. There should be no difficulty in distinguishing in these tracings the marks of the auricular and ventricular contractions. Before the onset of the attacks the block was complete; the auricles and ventricles had perfect and independent rhythms. The main feature of the attacks is the marked slowing of the ventricular rate; at the time of maximum slowing the interval between two ventricular beats may be more than ten seconds. It may be seen that during these intervals the auricles continue to beat with perfect regularity.

[25] Gaskell, *Schafer's Text-book of Physiology*, 1900, ii, 172.
[26] *Ibid.*, p. 190.

An exact analysis of the events of the attack illustrated in Fig. 12 is given in Table XII. In this it may be seen that the ventricular rate slows down a trifle before the longest period of *asystole* occurs. After this period the ventricular rate gradually increases until it becomes faster than before the onset of the attack. The changes in the auricular rate are just the reverse of these. Synchronously with the slowing of the ventricular rate there is a slight but definite acceleration of the auricular rate. During the attack the auricular rate is decidedly accelerated, but as the attack passes off the auricular rate decreases and when recovery has become complete the auricular rate is slower than before the onset of the attack.

TABLE XII.

ANALYSIS OF SYNCOPAL ATTACK (JANUARY 7, 1905).

No. of Vs.	Duration of Vs. in $\frac{1}{5}$ Sec.	Duration of undeformed As. in corresponding V. cycle in $\frac{1}{5}$ Sec.
1	14 (?)	3.6 – 3.4
2	12.6	3.4
3	13.4	3.5 – 3.4
4	14.0	3.4 – 3.3
5	14.2	3.4 – 3.2
6	52.6	3.4 (?) – 3.2 – 3.2 – 3.1 – 3.1 – 3 – 3 – 3 – 3 – 3 – 2.8 – 2.8 – 2.8 – 2.6 – 2.8 – 2.8 – 2.8 – 2.8
7	26.2	2.8 – 2.6 – 2.6 – 2.6 – 2.8 – 2.8 – 2.8
8	16.6 (?)	2.4 – 2.5 – 2.8 – 3.4 (?)
9	12.4	3 – 3
10	11.4	3 – 3
11	11.6	3.2 – 3.2
12	11.4	3.2.
13	11	3.6.
14	10.8 (?)	4 (?)
15	11	4.2
16	11	4.2
17	11	4.2
Some time later.	12.4	3.6 – 3.6.

Careful analysis of the tracings obtained during the course of other attacks shows that exactly the same events occur in all (see Table XIII).

The Cause of the Slowing of the Ventricular Rate which is Associated with the Attacks of Syncope.—It has been stated that the reduction of the ventricular rate which occurs during the

attacks is always accompanied by an increase of the auricular rate. The first step in the solution of this problem, therefore, is to determine what significance, if any, this association has.

In the first place this association demonstrates that the ventricles are not slowed by an increase in inhibition caused by the arrival in the heart of impulses transmitted through the vagi. For if this were the case the auricular rate too would almost certainly be slowed.[27] This factor having been excluded, three possible explanations suggest themselves.

(a) The increase of the auricular rate and the decrease of the ventricular rate are caused by one and the same thing, perhaps by a local irritation of some kind. No suggestions can be offered in support of this idea.

(b) The increase of the auricular rate is in itself the cause of the slowing of the ventricular rate. It will be shown later, indeed, it has already been stated, that anything which brings on an attack first increases the auricular rate. It has also been shown that by increasing the auricular rate a partial block may be made more complete. But how can the beats of the auricles affect the ventricles when the auricular impulses presumably do not reach the ventricles? The only suggestion that we can offer in answer to this question is one which cannot be tested. It is this: When the block is complete the ventricles beat of their own accord. In order that the contractions of the ventricles may be co-ordinated it is possibly essential that the impulses start at one point. Upon the rhythm of this place the rate of the ventricles must depend. Now it is possible that although the auricular impulses do not cause the ventricles to contract, they may still have some effect upon the *organ* which regulates the ventricles. And an increase of the auricular rate may so affect this *organ* as to disturb its rhythm.

(c) The decrease of the ventricular rate is the cause of the

[27] Pawlow (*Arch. für Phys.*, 1887, 498) has shown that the heart reacts differently to stimulation of the several cardiac nerves, and it is therefore possible that the vagus centre may exercise a special control over each chamber of the heart. That this is the case has not, however, been demonstrated.

increased auricular rate. At first sight this idea seems to be the most plausible one. The decrease of the ventricular rate, by diminishing the blood supply to the medullary centres, might lower the tone of the inhibitory centre and so cause the acceleration of the auricles. But there is a serious objection to the acceptance of this view. The changes in rate are simultaneous; in the opinion of the author, the time which intervenes is not sufficient to allow of the completion of a reflex or of anæmia of the centre. Still, it might be assumed that the force of the ventricular contraction decreases before the rate of contractions is materially affected. This can be tested. For if it is the case, the onset of the attacks should be preceded by a fall of blood-pressure. A study was therefore made of the blood-pressure variations associated with the attacks.

The Relation between the Blood-Pressure and the Slowing of the Ventricles in Syncopal Attacks.—As the attacks begin without warning, and as they are usually over within the time that is required to make a blood-pressure estimation by usual methods, great difficulty was experienced in obtaining satisfactory results. Many blood-pressure estimations were made with the blood-pressure instrument devised by the author, using for this purpose the most rapid method, that of continuous escapement.

Many attacks occurred while the estimations were being made, but nothing indicating a constant change of the blood-pressure just preceding an attack could be discovered. The records, however, show clearly the fall of pressure which is naturally associated with the slowing of the ventricular rate. Thus it may be seen in Fig. 15 that whenever an attack occurred while the pressure on the arm exceeded the minimum intravascular pressure, the amplitude of the oscillations diminished; and conversely, whenever an attack occurred while the pressure on the arm was less than the minimum intravascular pressure, the amplitude of the pulsations increased.

The basis for this interpretation, and for the interpretation of what follows is intimately connected with the principle employed in estimating blood-pressures with the author's instrument. According to Howell and Brush, maximal oscillations of the

arterial wall, and consequently of the recording mechanism of the instrument used, are obtained when the pressure on the artery is equal to the minimum or diastolic intra-arterial pressure.[28] Consequently if we put upon the artery a pressure which exceeds the intravascular pressure, and if we then allow the pressure on the arm to fall slowly, the amplitude of the pulsations will increase until the extravascular pressure falls below the intra-arterial pressure, when the amplitude diminishes more or less abruptly. Conversely, if we put upon the artery a constant pressure which lies between the maximum and minimum arterial pressures, then any rise of the arterial pressure, by bringing the intravascular minimum closer to the extravascular pressure, would increase the amplitude of pulsations; and any fall of the arterial pressure, by increasing the difference between the intra- and extra-arterial pressures, would diminish the amplitude of pulsations.

It may be seen in Fig. 16 that there is a very decided change in the amplitude of oscillations in the region which lies between the places marked 119 and 85. In this region a change of pressure equal to 5 mm. Hg. produces a decided change in the amplitude of the oscillations. We therefore made a continuous record with a constant pressure of 100 mm. Hg. on the arm (Fig. 17). While these tracings were being made the patient had a few mild attacks. It may be seen in the figure that slight oscillations of the pressure were constantly in progress; but no constant relation can be detected between these waves of pressure and the onset of ventricular asystole. We may therefore state positively that the increase in the auricular rate, which is always synchronous with the slowing of the ventricular rate, is not dependent upon blood-pressure changes caused by changes in the force of the ventricular beats which might precede the slowing of the ventricular rate.

[28] Howell and Brush, Mass. Med. Society, June 12, 1901. The blood-pressure tracings of this patient show very conspicuously the first and second maxima that obtain while making the estimations by the method of continuous escapement (Erlanger, *Johns Hopkins Hosp. Reports*, 1904, xii, 53). The first maximum is here higher than the second; this is, however, not the case when the method of intermittent escapement is used.

The Relation between Asphyxia and Variations of the Ventricular Rate.—The effect of asphyxia on the rates of the auricles and ventricles in heart-block has been described in another connection. There it was stated in passing, that one of the late effects of holding the breath is a diminution in the rate of the ventricles. In that place, only those experiments were presented in which the diminution in the rate of the ventricles was slight. Here it will be shown that the resulting diminution of the rate of the ventricles may be so marked as to give the picture seen in typical attacks.

One of the tracings (Fig. 12; see also Table XII) which was used to illustrate the events of a typical attack was made while the patient was holding his breath just after having taken moderate muscular exercise. Two other tracings have been obtained of attacks brought on accidentally by the same combination of events. We did not deem a repetition of such an experiment justifiable. But this observation has been almost certainly confirmed by events occurring in the natural course of the disease.

At one time the patient developed a mild form of Cheyne-Stokes respiration. At this time typical attacks, with marked slowing of the rate of the ventricles, recurred with great regularity (see Table XIII). The slowing of the ventricular rate usually began early in the period of dyspnœa, just at the time when, in other conditions, the heart-rate begins to increase. The auricles show this increase in rate, but, as has always been the case, the ventricular rate slows when the auricular rate begins to increase.

It is therefore not possible to determine whether it is the asphyxia, or the increase of the auricular rate, which causes the ventricular rate to diminish. But we believe that it will eventually be determined that the latter phenomenon is the causative factor.[29]

[29] Holberton (*Medico-chirurgical Trans. of the Roy. Soc.*, 1841, xxiv, 76) seems to have been the first and only investigator to have described this association. In an account of a typical instance of Stokes-Adams disease, he says: ". . . . when he was free from excitement or casual disorder the pulse was thirty-three, but it was easily altered. Mental excitement usually increased it, and, in general,

The Effect of Atropine on the Frequency of the Attacks.—It has been stated that the first experiment performed to test the action of atropine was begun at a time when the patient was having mild attacks at frequent, although irregular, intervals. He had a mild attack about one minute after the atropine was administered, and during the succeeding minutes slight ventricular slowing occurred occasionally. But after this had passed off, the patient had no attacks until late in the evening, about six hours after the atropine had been given. The attacks then returned and continued with undiminished frequency throughout the next day.

The fact that atropine increases the rate of the auricles but still does not cause slowing of the ventricular rate, might, at first sight, appear to be inconsistent with the view that the attacks are caused by an increase of the auricular rate. But analysis of the attacks shows that the slowing of the ventricular rate is usually associated with a rather sudden increase of the auricular rate. The increase of the auricular rate which is caused by atropine is very gradual, and, by removing the auricles from the influence of the vagi, at least one of the factors which cause sudden variations in the rate of the auricles is annulled. This fact might account for the freedom from attacks after the administration of atropine.

The Time Relation between the Subjective Symptoms and the Cardiac Signs of an Attack.—In many experiments, while making tracings, we had the patient indicate by a movement of his hand the moment at which he felt certain that an attack was imminent. At this instant a mark was made on the moving drum. Each time it was found that the ventricles had begun to slow down several seconds before the patient was aware of the approach of an attack (see Fig. 17). This observation is in accord with the results of all other investigators who have studied this relation with care.[30] It indicates that the attacks of syncope are

this was followed by a corresponding slowness of the pulse, and often by a fainting fit; and a sudden rise of the pulse, or even a gradual increase above the point that might in his state be called his healthful standard, usually indicated mischief and was found to be a bad symptom."

[30] See Webster, *Glasgow Hosp. Reports*, 1900, iii, 413.

TABLE XIII.

ANALYSIS OF ATTACKS DURING CHEYNE-STOKES RESPIRATION.*

Periods of dyspnœa.	No. of Vs.	Duration of Vs. in ⅛ Sec.	Duration of undeformed As. in corresponding V. cycle in ⅛ Sec.
	1	11.8	2 in 5.5 = 2.75
	2	11	2 in 5.8 = 2.9
	3	10.8	2 in 6 = 3
	4	10.4	3.2
	5	10.5	3.5
	6	10.5 −	3.6
	7	10.5 −	3.6
	8	10.4	3.5
	9	10.6	(?)
	10	10.4	3.6
	11	10.5	(?)
	12	10.8	3.5
	13	11	3.3
	14	11.2	2 in 6.6 = 3.3
	15	11.6	2 in 6 (?) = 3
	16	13.8	3 in 9.2 = 3.1 +
	17	32.2	2.8 + −2.8−3−2.8−2.8−2.8−2.8−2.8
	18	18	2 in 5.5 = 2.75
	19	17	5 (?) in 12.8 = 2.56
	20	15.8 (?)	2 in 5.5 = 2.75
	21	16 (?)	4 in 10.6 = 2.6
	22	15.5	4 in 11 = 2.75
	23	14.5	3 in 8.5 = 2.8
	24	12	2 in 6 = 3
	25	11.5 (?)	2 in 6 = 3
	26	11.5	3
	27	11	(?)
	28	(?)	(?)
	29	11	3.5
	30	10.8	3.6
	31	10.8	2 in 6.9 = 3.4
	32	10.8	2 in 6.4 = 3.2
	33	11	3.4
	34	11.2	2 in 6.2 = 3.1
	35	11	2 in 6.2 = 3.1
	36	11.6	(?)
	37	11.6	(?)
	38	12.4	2 in 6 (?) = 3
	39	13	2 in 5.8 = 2.9
	40	12.2	2 in 5.6 = 2.8
	41	12	(?)
	42	11.8	2 in 6 = 3
	43	11.6	3.2
	44	11.8	3.2
	45	11.5	3.2
	46	11 +	2 in 6.8 = 3.4
	47	11.4	3.2
	48	11.6	3.5

* No tracing was made of the respirations, but the periods of dyspnœa are as a rule clearly marked by respiratory waves on the cardiogram. In many places the respiratory movements were so violent as to make the cardiogram illegible.

TABLE XIII.—*Continued.*

Periods of dyspnœa.	No. of Vs.	Duration of Vs. in $\frac{1}{8}$ Sec.	Duration of undeformed As. in corresponding V. cycle in $\frac{1}{8}$ Sec.
	49	11.6	3.4
	50	11.6	3.6
	51	12.8	2 in 6.8 = 3.4
	52	13.5	2 in 6.6 = 3.3
	53	52.8	3.2 − 3 − 3 − 3 − 3 − 3 − 3 − 2.8 − 3 − 3 − 3 − 3 − 2.8 − 2.8 − (?) − (?)
	54	22	(?) (?)
	55	17	3 in 8 = 2.7
	56	15.5	3 in 8 = 2.7
	57	12	(?)
	58	11.7	3
	59	11	2 in 5.5 = 2.7
	60	10.8	2 in 6.2 = 3.1
	61	10.5	2 in 6.6 = 3.3
	62	10.4	3.4
	63	10.5	3.6
	64	(?)	3.8
	65	(?)	3.6
	66	10.6	3.4
	67	10.5	3.5
	68	11	3.4
	69	11	3.5 −
	70	11.4	3.2
	71	12.4 (?)	2 in 6.2 = 3.1
	72	13.6	3
	73	13.5	3 in 8.5 (?) = 2.8
	74	14.2	2 in 6 = 3
	75	14.6	2 in 5.6 = 2.8
	76	14	2 in 6 = 3
	77	13	2 in 6 = 3
	78	12	2 in 6 = 3
	79	11	2 in 6.2 = 3.1
	80	11.2	3.4
	81	11.4	3.4
	82	11.4	3.4
	83	11.2	3.6
	84	11	3.5
	85	11+	3.4
	86	11.5	3.5
	87	11.6	2 in 6.8 = 3.4
	88	12	3.2
	89	14	3 in 9.5 = 3.2 −
	90	34.6	2 in 5.4 = 2.7 and 2 in 5.4 = 2.7
	91	17.6	(?)
	92	16.4	(?)
	93	15	2 in 5 = 2.5
	94	12.5	(?)
	95	12	3
	96	11	3
	97	10.6	2 in 6.4 = 3.2
	98	10.6	3.4
	99	10.6	(?)
	Etc.		

the result of a deficient blood supply to the brain. It is, of course, possible that the phenomenon is actually primary in the medullary centres, and that the unconsciousness comes on only after a central irritation has slowed the rate of the ventricles. So far as we are aware a slowing of the rate of the ventricles caused by central irritation could come through the inhibitory apparatus only. But irritation of the vagus centre would slow the auricles as well as the ventricles. As this does not occur it may be stated positively that *the cause of the syncopal attacks lies in the heart and not in the medullary centres.*

The Relation between the Frequency and the Severity of the Attacks and the Degree of Completeness of the Block.—We have obtained no exact data bearing upon this relation. However the following observations may be of interest, although it remains for future investigations to determine their value.

During the first few weeks of our observation of this case the heart-block was always complete. During this period the patient had but one attack of syncope while under observation. For this one we were directly responsible: it came on while the patient was holding his breath after having taken rather severe muscular exercise.

Later in the course of these observations the block frequently became partial with a 3:1 rhythm or even, occasionally, with a 2:1 rhythm. On the first day upon which the rhythm occasionally became 2:1, the ventricular rate while the rhythm was 3:1 was 39.2 beats per minute, the fastest we had yet seen, and the auricular rate was 78.3 per minute, the slowest we had yet observed. Still, on this day the patient had attacks more frequently than was usual. He stated that the attacks began to appear at about 2:00 A.M. and had occurred frequently throughout the rest of the night. During the morning hours he had the most severe attack that occurred during his stay in the hospital. At about 2:00 P.M. the patient went to bed. At this time it was found that the block had become complete, with a ventricular rate of 31 per minute and an auricular rate of 84.6 per minute. The patient then remained in bed for several days. During this time the block was always complete. The attacks recurred with

increasing frequency so that finally they occurred every few minutes. The patient's condition then gradually improved, although the block remained complete.

The only conclusion warranted by these observations is that the increased severity and frequency of the attacks which occurred while the block was partial, was possibly the result of some condition which tended to produce complete block. The condition of partial block was probably not in itself responsible for the attacks.[31]

The Relation between the Blood-pressure and the Frequency of the Attacks.—It has been shown that the attacks are not ushered in by any perceptible change in the blood-pressure. But it might be considered possible that although the individual attacks are not caused by changes in the blood-pressure, the general level of the blood-pressure might have something to do with the frequency of the attacks.

TABLE XIV.

BLOOD-PRESSURE ESTIMATIONS MADE WHEN SYNCOPAL ATTACKS WERE INFREQUENT. BLOCK COMPLETE.

Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
120	60	115	62	115	62	117	57	120	58
115 −	62	(?)	62	115 +	65	115 (?)	58	122	62
115	60	120	60	117	65	120	58	120	62
115	60	(?)	62	117	60 +	125	58	(?)	62
117	60	116	60	117	62	125	60	120	60
112	65 −	115	60	117	62	125	58	120	64
115	60 −	120	60	118	64	122	62 −	(?)	60 +
115	60	117	62	120 −	62	(?)	58	120	60
115	58	117	60	115	60	118	64		
115	60	117	64 −	117	58 +	122	60	118	61

The long list of blood-pressure estimations given in Table XIV contains the results which were obtained on a day when the attacks were rather infrequent. They were so infrequent that none occurred while making blood-pressure estimations every five minutes during many hours. Two days later the patient was having attacks every few minutes. On this day frequent

[31] See Holberton, *loc. cit.*

estimations of the blood-pressures were again made but the attacks recurred so rapidly that at least one occurred in almost every blood-pressure estimation (see Fig. 15). This made accurate estimations of the blood-pressure exceedingly difficult. This difficulty was in part overcome in the following way. The writing point of an electromagnetic chronograph was arranged so as to write immediately beneath the lever of the sphygmomanometer. A key, placed in the circuit of the chronograph, was closed with the hand each time the mercury column in falling passed a 5 mm. mark. In this way a record was made of the pressure at which practically each of the pulsations was obtained. But even with the aid of this device, judgment of the pressures was difficult. The difficulties are illustrated in the table (No. XV). Comparison of the results included in this table with those given in the preceding table seems to indicate that the blood-pressure was perhaps a trifle higher on the day when the attacks were frequent. The difference is however so slight that no positive conclusion is warranted. Indeed, it is possible that the higher blood-pressure seen here might have been the result of an insufficient action of the heart (asphyxia), rather than the cause of the greater frequency of the attacks.

BLOOD-PRESSURE VARIATIONS DURING THE COURSE OF THE DISEASE.

In this section will be given only the average results of blood-pressure estimations which were made while the heart-block was complete and after complete recovery. For, if a change of blood-pressure is associated with each change in rhythm, this should be most clearly illustrated in such a comparison. From the results of such a comparison the blood-pressures during intermediate stages may be inferred. The only objection to this method consists in the long interval of time which must necessarily intervene between the two sets of observations. This objection has, in part, been met by comparing a large number of observations.

In this way it has been determined that with the change from complete block to the normal state, the maximum blood-pres-

sure probably falls slightly—from 118 to 111; whereas the minimum blood-pressure rises somewhat more—from 61 to 74. The pulse-pressure therefore decreases from 57 to 37.

TABLE XV.

BLOOD-PRESSURE ESTIMATIONS MADE WHEN SYNCOPAL ATTACKS WERE FREQUENT. BLOCK COMPLETE. NOS. 1–22 WERE MADE BEFORE ADMINISTRATION OF ATROPINE; 23–29 ON DAY AFTER

No. of Estimation.	Max. Pres.	Remarks.	Min. Pres.	Remarks.
1	120–125	No V. slowing	?	V. slowing
2	110–115	Slight V. slowing	65–70	Slight V. slowing
3	115–120	" " "	55–60	V. slowing
4	120	No V. slowing	65	No V. slowing
5	120–125	" " " (?)	60–65	" " "
6	105–110	V. slowing	65–70	" " "
7	125–130	No V. slowing	70	" " "
8	125	" " "	60–65 (?)	(?)
9	105	V. slowing	60–65 (?)	No V. slowing
10	120–125	No V. slowing	65	" " "
11	115+	" " "	60–65	Slight V. slowing
12	120	Preceded by V. slowing	65	No V. slowing
13	105	V. slowing	65–70+	" " "
14	120	No V. slowing	65	" " "
15	120–125	Preceded by V. slowing	?	V. slowing
16	120	No V. slowing	?	" "
17	120	" " "	70–	Preceded by V. slowing
18	105–110	V. slowing	65	No V. slowing
19	110–115	Preceded by V. slowing	65–70	" " "
20	115–120	Followed by V. slowing	70 (?)	Preceded by V. slowing
21	125–130	Just preceded by V. slowing	65–70+	No V. slowing
22	115–120	" " " " "	65–70	" " "
23	112	Slight V. slowing	60+	No V. slowing
24	115	" " "	65	" " "
25	122	No V. slowing	?	V. slowing
26	125	" " "	?	" "
27	120	Preceded by V. slowing	—	
28	120	No V. slowing	62	No V. slowing
29	119	" " "	63	" " "

It is a well established fact that the mean blood-pressure is much closer to the minimum pressure and follows its variations much more closely than those of the maximum pressure. The conclusion is therefore warranted that the blood-pressure is somewhat higher when the heart is normal than when complete heart-block exists.

This result affords the opportunity of emphasizing the futility of drawing conclusions as to blood-pressure variations from the knowledge of the maximum pressure alone in conditions in which he blood-pressure variations are not marked and in which variations of the heart-rate or of the force of the heart-beat are involved. Had only maximum blood-pressure estimations been made in the study of this case, we would have arrived at the illogical and erroneous conclusion that the blood-pressure is lower when the heart is beating with its normal rate than when the ventricle beats but twenty-four times per minute. As a matter of fact this was the conclusion to which the ward records of the maximum pressure had led.

THE RELATION BETWEEN HEART-RATE AND RESPIRATORY RATE.

While the heart-block was complete all tracings in which the respirations and apex-beat were recorded simultaneously show a remarkable tendency to a synchronism between respiratory and ventricular rhythms. When the block is complete inspiration as a rule begins with the beginning of ventricular relaxation and lasts somewhat less than one half of one cardiac cycle (see Fig. 18).

When the rhythm is 3 : 1 there is, as a rule, one respiratory cycle for two ventricular cycles. Here the beginning of inspiration usually coincides with about the middle point of a ventricular cycle. When, for any reason, the respiratory rate is momentarily disturbed, it usually soon falls into cadence with the ventricular rhythm. This synchronism has been mentioned merely on account of the interest which attaches to it; no attempt has been made to determine its cause.

SECOND CASE OF STOKES-ADAMS DISEASE.

The following is the history of another case of Stokes-Adams disease which has come under the author's observation.

R. H. S., colored, aged thirty-eight, was admitted into the out-patient department of the Johns Hopkins Hospital on November 12, 1901, complaining of "misery in the pit of the stomach and dizziness." The patient was not seen again until April 8, 1905, when he was readmitted, complaining of shortness of

breath, vertigo, and pain in the back. There are many discrepancies in the histories obtained at each of his admissions, but the essential facts are probably these:

His family history is negative. In his personal history it is perfectly clear that he had gonorrhœa and syphilis. The latter was acquired about two years before the patient's first admission. He took anti-syphilitic treatment for about four weeks.

Five weeks before his first admission the patient was taken with dizziness and shortness of breath. "Things swim before him so much at times that he falls, but he has never lost consciousness." The pulse at this time was thirty-four to the minute. The heart sounds were clear. The patient informs me that seven months elapsed after first coming under observation before definite syncopal attacks made their appearance.

At the time of his second admission the patient stated that he was having attacks of vertigo at the rate of about four per week. The pulse-rate was then thirty-two per minute. Soft murmurs were associated with each of the heart-sounds. The auricular pulsations were nowhere audible, but it was possible to determine the auricular and ventricular rates by counting the waves in the veins of the neck.

But one opportunity was afforded the author of making tracings of the movements of the heart. The cardiogram obtained shows all of the features seen in the first case while the heart-block was complete. The rates of the auricles and ventricles determined by measurement of the tracing are 31.7 and 89.3 per minute, respectively.

SUMMARY OF PART I.

The investigation of a typical case of Stokes-Adams disease has shown that the symptoms of this case undoubtedly are caused by some lesion in the heart which gives rise to the condition now generally termed heart-block. Practically all degrees of heart-block have been observed, namely: complete heart-block, and partial block with 4:1, 3:1, and 2:1 rhythms, and occasional ventricular silences. These stages occurred during recovery, probably under the influence of anti-syphilitic treatment, recommended by Dr. Osler.

Experiments testing the reaction of the heart to various extrinsic influences demonstrate that when the block is complete the ventricles do not respond to influences presumably of vagus origin, although the auricles still respond normally to such influences; that effects exerted upon the heart presumably through the accelerators still influence the rate of the ventricles as well as that of the auricles.

When the block is partial the rate of the ventricular contractions varies proportionally with the rate of the auricular contractions, but only within certain limits. When these limits are exceeded the block becomes more complete, *i. e.* a 2:1 rhythm may be changed into a 3:1 rhythm, this into a 4:1 rhythm, and this into complete block, and *vice versa*.

The syncopal attacks are, in all probability, directly dependent upon a marked reduction of the ventricular rate. Such reductions of the ventricular rate are always associated with an increase of the auricular rate, and it is believed that the latter is the cause of the former.

The *mean* blood-pressure is higher when the heart-beat is normal than when complete block exists.

EXPLANATION OF PLATES XXXII–XXXV.

Fig. 1.—Cardiogram (uppermost), jugular sphygmogram (middle) and, brachial sphygmogram (lowermost) during complete heart-block. Time = $\frac{1}{5}$ sec.

Fig. 2.—Same as Fig. 1.

Fig. 3.—Pantogram of Fig. 1. For descriptions of reconstructions, see p. 685.

Fig. 4.—Brachial sphygmogram during complete heart-block.

Fig. 5.—Brachial sphygmogram made while the pressure on the artery was falling from a point above the systolic pressure to a point below the diastolic pressure.

Fig. 6.—Cardiogram during 4:1 rhythm.

Fig. 7.—Cardiogram during 3:1 rhythm.

Fig. 8.—Cardiogram during 3:1 rhythm showing at the end a gradual lengthening of the intersystolic period associated with the development of complete block.

Fig. 9.—Cardiogram showing one 4:1 cycle in the midst of 3:1 cycles.

Fig. 10.—Cardiogram during 2:1 rhythm.

Fig. 11.—Cardiogram during normal sequence.

Figs, 12, 13, and 14.—Cardiograms during syncopal attacks.

Fig. 15.—Tracings made while determining the blood-pressure by the method of continuous escapement, showing the effect of syncopal attacks on the blood-pressure. The marks on the base line indicate decrements of 5 mm. Hg.

Fig. 16.—Tracing made while determining the blood-pressure by the method of continuous escapement.

Fig. 17.—Tracing made with the sphygmomanometer with a constant pressure on the arm to determine whether or not the attacks are ushered in by a change in the blood-pressure. The crosses were made at the moment the patient noted the first symptoms of the attacks.

Fig. 18.—Tracings of the respiratory movements and apex-beat (lower) during complete block.

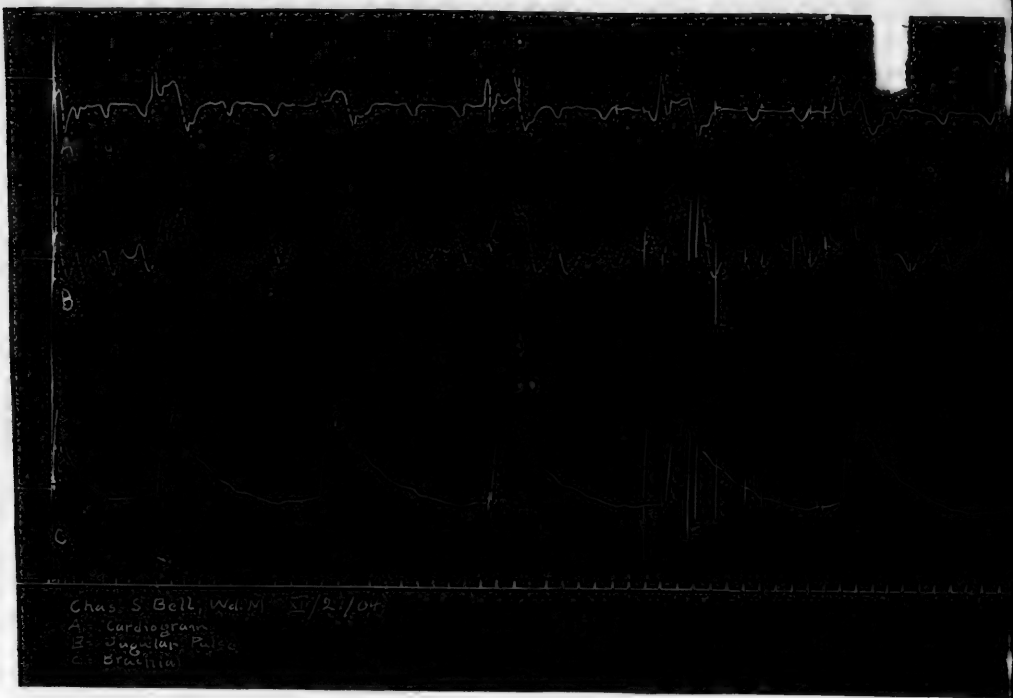

A
B
C
Chas. S Bell, Wd. M XI/2/04
A. Cardiogram
B. Jugular Pulse
C. Brachial

FIG. 1.

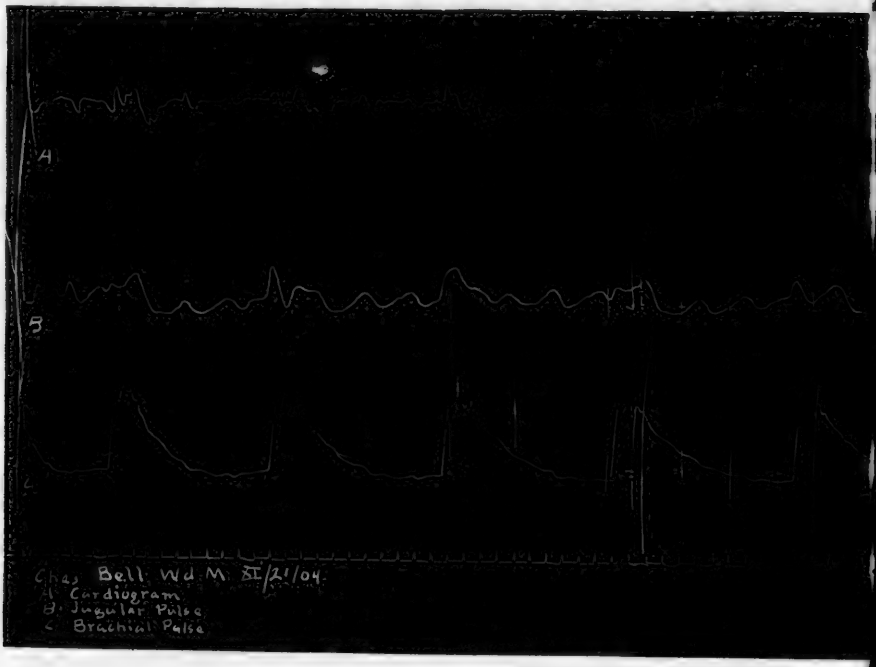
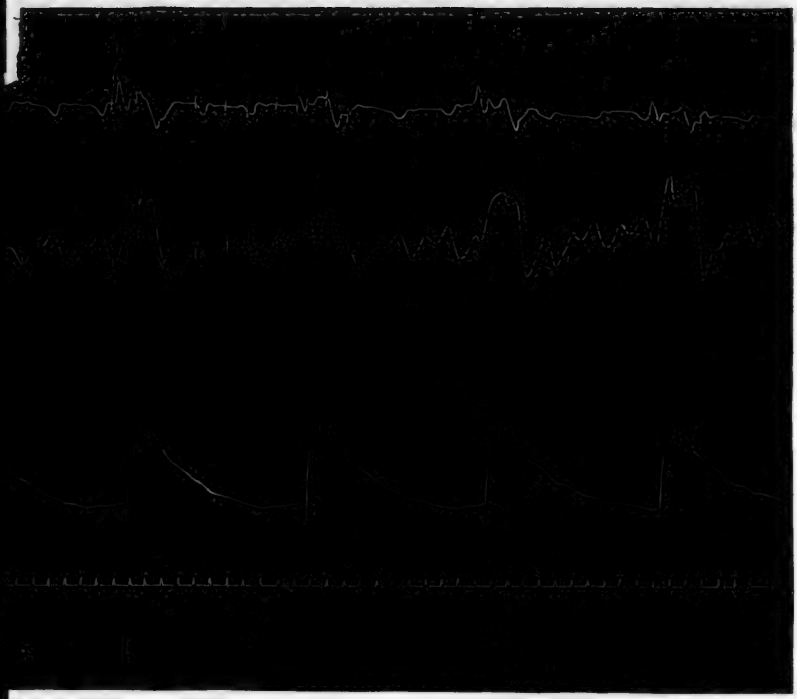

A
B
C
Chas Bell Wd M XI/2/04
A Cardiogram
B. Jugular Pulse
C Brachial Pulse

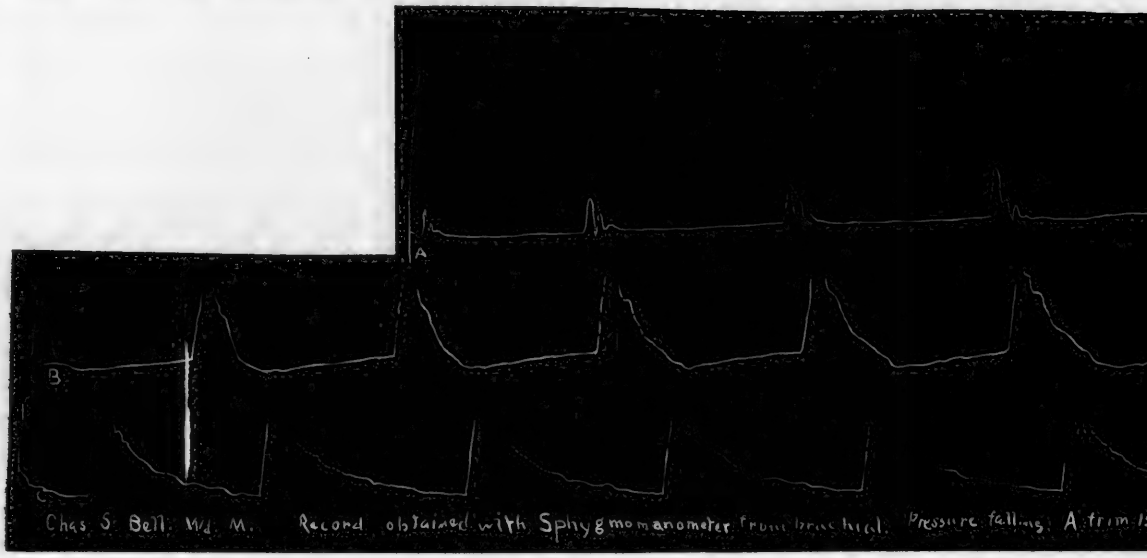
FIG. 2.

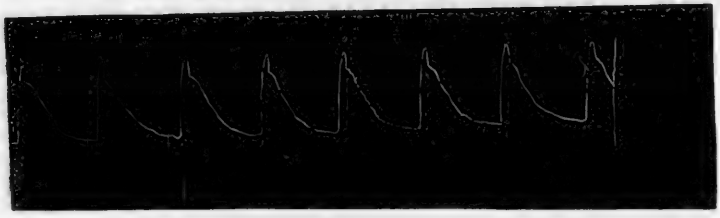
PLATE XXXII.



Reconstructed ventricular cardiogram.
Original cardiogram.
Reconstructed auricular cardiogram.
Original jugular sphygmogram.
Reconstructed jugular waves of auricular origin.
Reconstructed jugular waves of ventricular origin.
Brachial sphygmogram.

FIG. 3.


A
B
Chas. S. Bell. M.d. M.
Record obtained with Sphygmomanometer from brachial.
Pressure falling.



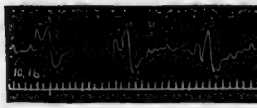


FIG. 4.

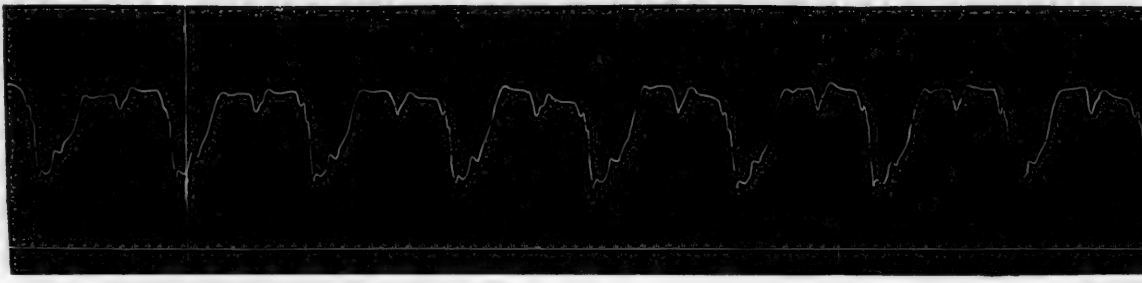


FIG. 8.

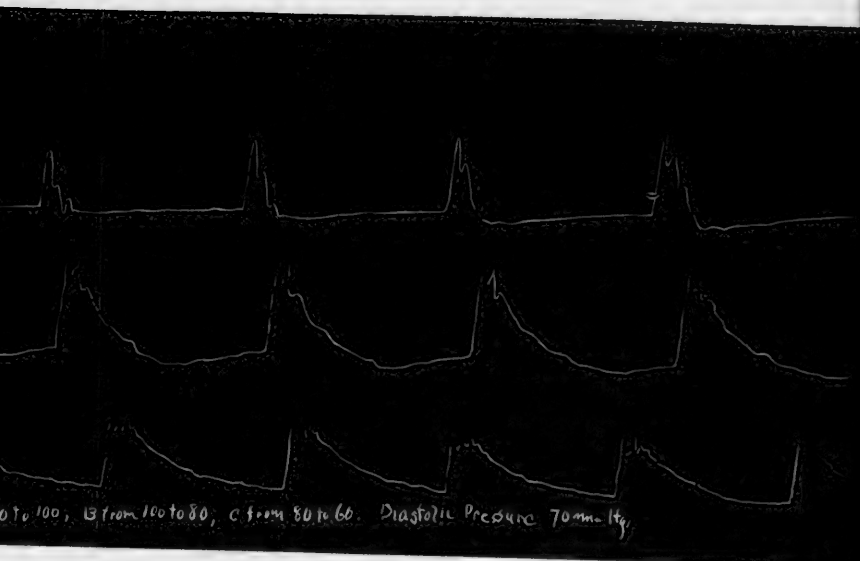
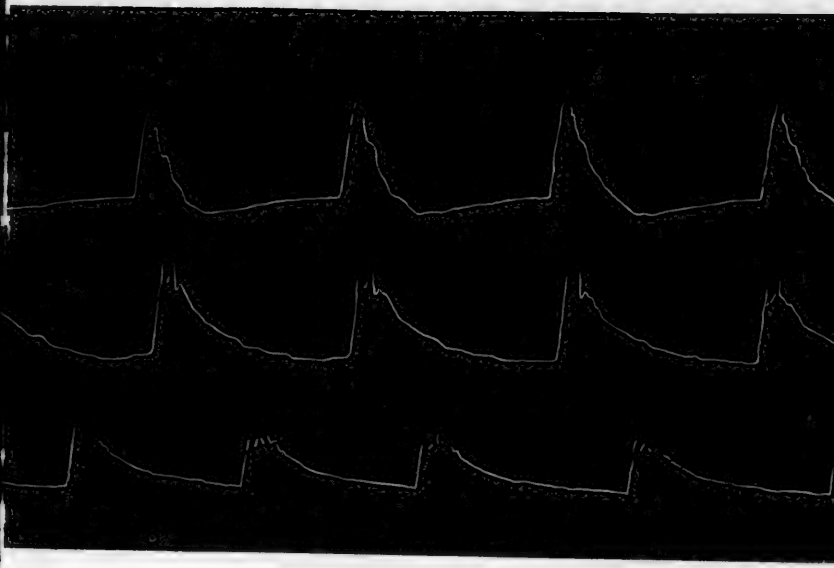

0 to 100, B from 100 to 80, C from 80 to 60. Diastolic Pressure 70 mm. Hg.

FIG. 5.

FIG. 6.



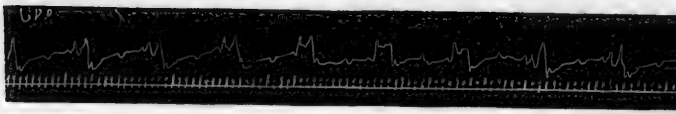
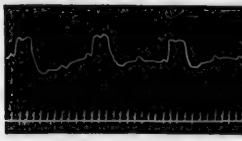
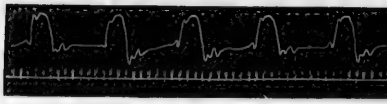
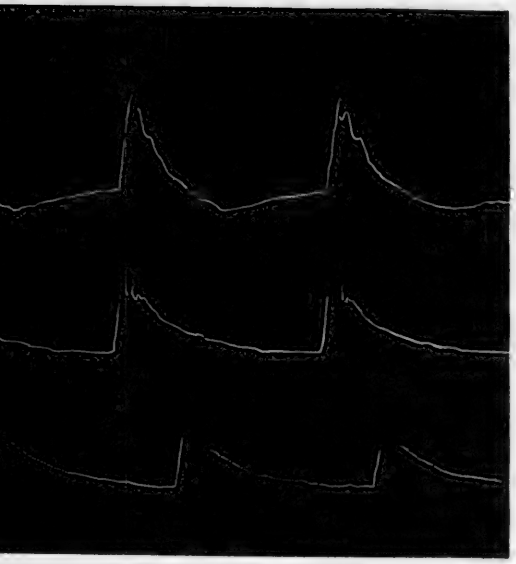


FIG. 7.







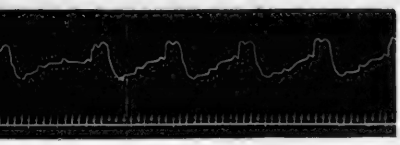


FIG. 9.

FIG. 10.

THE ACTION OF GLYCERIN ON BACTERIA IN THE PRESENCE OF CELL EXUDATES.

By J. J. KINYOUN, M.D., Ph.D., Philadelphia.

Glycerin has been employed for a number of years for the conservation of vaccine virus[1] and other materials of similar nature, *e. g.*, the virus of rabies, clavele, rinderpest, and foot and mouth disease. Its preservative properties for animal and vegetable tissues are also well recognized. It has, however, been employed more as a diluent, particularly in the case of bovine virus, than because of its other properties, as it seemed better adapted for this purpose than the other substances tested.

Not only does glycerin possess the property of conserving the activity of vaccine virus for a longer time than any other preservative agent tested, but it has also a marked influence on the bacterial content of the virus. Leoni[2] claims to have discovered this fact as long ago as 1889, and since then he has continually employed glycerin for destroying the bacteria in, as well as for the preservation of, the virus. Notwithstanding Leoni's claim of priority, we have until now given Copeman[3] the credit of pointing out the value of glycerin as a preservative and germicide when added to vaccine virus. Copeman's statements regarding the action of glycerin on the bacteria present in the virus have been accepted by nearly all, if not by all, as final, and have doubtless been the cause of many placing too much confidence in it as a purifier of vaccine, as Rosenau[4] as a result of his study of the subject has already stated.

[1] Cheyne, R. R., "On the Preservation of Vaccine Lymph," *Medical Times* (London), 1850, xxi, 267.

[2] Leoni, O., *Sui fattori dell' activita pattogena e specifica de vaccino. Trans. XIth. Session Internat. Medical Congress;* Section on Hygiene, 1894, p. 31.

[3] Copeman, *Vaccination*, 1899, pp. 151–182.

[4] Rosenau, *The Antiseptic and Germicidal Properties of Glycerin*, Hygienic Laboratory, U. S. Public Health and Marine Hospital Service, Bulletin No. 16, 1902.

During the latter part of 1902, and as a part of a discussion of the subject before the Society of American Bacteriologists, I showed a few plate cultivations made from vaccine emulsion treated with glycerin and other substances. The observations made at this time suggested that perhaps there were other factors than the glycerin concerned in the destruction of the bacteria present in the glycerinated virus. With this possibility in view the writer has continued this study and has accumulated additional facts which would seem to add some little to our knowledge of the subject.

The addition of glycerin to vaccine virus in the proportion of from 50 to 60 per cent. of its volume will, as a rule, cause a gradual diminution in the number of the bacteria present, but the effect at best is variable. The glycerinated vaccine emulsion shows, as a rule, only a few living bacteria after being kept for two or three weeks at a low temperature; but if the mixture has been kept at room temperature or at a higher temperature, the diminution in number of the bacteria is proportionately greater.

On the other hand, it occasionally happens that another specimen of virus contains a comparatively small number of bacteria of a species which is usually readily destroyed by the glycerin, and yet these may persist for so long a time in a living state in the virus that its activity will be greatly impaired by the long contact with the glycerin and it thus be rendered unfit for use.

After encountering several such refractory vaccines, it became apparent that the employment of glycerin did not give uniform results, and, therefore, that the statement of Copeman regarding its action called for revision.

An emulsion of vaccine tissue, to which glycerin in the proportion of 50 to 60 per cent. of its volume has been added, will suffer a sudden drop in the number of bacteria it contains; but after this the further diminution in number may be slow and gradual as the following experiments show. Two specimens were employed, of which one (No. 1) only was tested against glycerin. The number of bacteria is given at 1-hour, 2-hour, and 24-hour periods. The temperature was 18–23° C.

Vaccine emulsion in water. .0001 c.c.		Vaccine emulsion in 50% glycerin. .0001 c. c.	
	I	II	I
Immediate	480	Innumerable	12,000
1 hour	600		1,920
2 hours	—	Innumerable	1,600
24 "	Innumerable		

Low temperatures, as stated, exert a marked influence on the rapidity with which glycerin destroys bacteria. Emulsions of vaccine in glycerin of 60 per cent. strength, kept in a refrigerator at about 10° C., gave the following counts on the days mentioned:

	1st day.	7th day.	14th day.	21st day.
No. of Sample 1.....	1,800	2,000	150	78
" " " 2.....	12,600	2,100	215	380
" " " 3.....	33,000	1,050	370	62
" " " 4.....	3,000	965	670	650
" " " 5.....	30,000	1,500	960	370
" " " 6.....	20,000	725	210	81
" " " 7.....	3,500	1,050	700	—
" " " 8.....	51,000	1,800	180	—

On making closer study of this phase of the subject it appeared that, as a general rule, the several varieties of bacteria were destroyed much more rapidly in vaccine emulsions containing glycerin alone than in cultures to which glycerin was added, or their suspensions in distilled water. The following table shows the viability of several varieties of bacteria subjected to various percentages of glycerin in bouillon and distilled water (page 728).

Bovine vaccine virus always contains a variety of bacteria. The species most commonly met with are the pyogenic cocci, the colon bacillus, B. vaccinalis variabilis, streptothrices, moulds, unidentified endogenous spore-bearing, bacilli, and, more rarely, the common air varieties.

All the experiments with vaccine and other substances enumerated in this paper were made at room temperature, which ranged between 12° and 23° C. The estimation of the number of bacteria in plate cultivation was made in fractions of a cubic centimetre. When an emulsion of vaccine tissue is made with distilled water, and the estimation of the bacteria is delayed a day, the number of bacteria increases so rapidly that it is next

to impossible to enumerate the colonies after that time, although dilutions of $\frac{1}{1,000,000}$ of a cubic centimetre are employed. On the other hand, if glycerin be added in quantity from 25 to 50 per cent. of the emulsions, the number of bacteria falls in a very short time to one half or less of the original number present.

		Dist. Water.	Bouillon.	Dist. Water.	Bouillon.	Dist. Water.	Bouillon.	Dist. Water.	Bouillon.	Dist. Water.	Bouillon.	Dist. Water.	Bouillon.
Percentage of Glycerin.....		60	60	50	50	40	40	25	25	12½	12½	6¼	6¼
Staph. Pyog. Albus.....		— 4	— 6	— 4	— 8	— 12	— 12	+ 28	+ 28	+ 28	+ 28	+ 28	+ 28
Strep. Pyog.....	"	— 2	— 2	— 4	— 4	— 4	— 4	— 4	— 6	— 18	— 24	+ 28	+ 28
B. Pyocyaneus.....	"	— 4	— 4	— 4	— 6	— 4	— 6	— 8	— 16	+ 28	+ 28	+ 28	+ 28
B. Prodigiosus.....	"	— 4	— 4	— 4	— 6	— 6	— 6	— 8	— 12	+ 25	+ 28	+ 28	+ 28
B. Swine Plague.....	"	— 2	— 2	— 2	— 2	— 2	— 2	— 2	— 4	— 2	+ 25	— 2	+ 25
B. Diphtheriæ.....	"	— 6	— 6	— 6	— 6	— 6	— 6	— 6	— 10	— 24	— 16	— 24	+ 28
B. Typhosus.....	"	— 4	— 4	— 6	— 6	— 6	— 6	— 8	— 12	— 14	+ 28	— 16	+ 28
S. Choleræ.....	"	— 2	— 2	— 2	— 2	— 2	— 2	— 2	— 6	+ 4	+ 8	+ 8	+ 10
B. Anthracis.....	"	— 135	— 135	— 157	— 140	— 157	— 157	— 160	— 160	— 200	— 160	— 164	— 200
B. Tetani.....	"	+ 27	+ 27	+ 27	+ 27	+ 27	+ 27	+ 27	+ 27	+ 27	+ 27	+ 27	+ 27
Rose Yeast.....	"	— 6	— 6	— 6	— 6	— 4	— 6	— 6	— 8	— 22	— 22	+ 27	+ 27
Aspergillus Niger.....	"	— 2	— 2	— 2	— 2	— 2	— 4						

NOTE.—The figures indicate days, the plus and minus signs indicate whether growth was or was not obtained on the corresponding day.

In view of the fact, which the previous table exhibits, that certain bacteria in pure culture are destroyed more slowly by glycerin than in vaccine and tissue emulsions, the notion occurred to me that the tissue fluids or cell contents might in some way be concerned in producing the sudden reduction in number, and also that this reaction might occur through some combination between

the glycerin and the constituents of the emulsion, or by virtue of the glycerin extracting from the cells some peculiarly active bactericidal substance.

Denys and LeClef[5] have shown that exudates caused by pus cocci are bactericidal to these organisms, and that the fluids extracted from such exudates also possess this destructive property; and Simon[6] claims to have proven that leucocytes obtained from aleuronat injections are decidedly bactericidal to streptococci and much more active than the fluid part of the exudate freed from cells, or the blood serum of the animal.

Emulsions of vaccine tissue made with water were taken as stock solutions. To these solutions were added equal quantities of immune sera as follows: Serum from a vaccinated calf, antidiphtheritic, antitetanic, antistreptococcic, antistaphylococcic, antipneumococcic, antidysenteric, and antityphoid sera; and also serum from a goat in which leucocytosis had been induced, and normal horse serum. The bacterial counts of 0.0001 cubic centimetre of the mixture were made at stated intervals, and they showed a reduction of the bacteria in nearly all the experiments during the first twenty-four hours, after which time rapid increase in number occurred. The reduction was greatest in those tests in which freshly drawn sera were used, and little or no reduction occurred when sera a week or more old were employed. The immune sera, except when fresh, did not exert as great bactericidal action as fresh normal serum; and their action was still weaker when the nomal serum was added to the emulsions before the immune sera. The removal of the cells from these sera, either by prolonged centrifugalization or by filtering through a Berkefeld filter, affected somewhat their bactericidal qualities.

With a view of ascertaining whether the addition of a heterologous exudate might influence the number of bacteria in watery vaccine emulsions, an exudate was secured from a rabbit twenty-four hours after injecting it subcutaneously with a sterilized suspension of aleuronat, and, after grinding to a fine paste, was

[5] Denys and LeClef, Sur le mécanisme de l'immunité chez le lapin vacciné contre les streptococoque pyogène. *Le Cellule*, etc., 1895, vol. xi, 188–190.

[6] Simon, *Centralblatt für Bakteriologie und Parasitenkunde*, 1901, xxix, 116–119.

added to the emulsions with an equal amount of water. The resulting mixture was rich in saprophytic and other bacteria. When equal quantities of these emulsions were mixed there followed, within twenty-four hours, a decided diminution in the number of bacteria. The controls of 0.0001 cubic centimetre gave innumerable colonies, whereas the number of colonies developing in the plates of the mixture was only from 300 to 750. The addition of a small quantity of fresh normal or immune serum caused a still greater reduction amounting, in extreme cases, to a fall to even as low as five colonies. The exudate obtained with aleuronat seemed to have a special action upon the pus cocci, as these were the first bacteria to disappear from the mixtures.

It was considered desirable to ascertain whether the serum from a sample of blood which contained an excess of leucocytes was more effective than normal serum in destroying the bacteria in vaccine emulsions. A medium-sized goat was, therefore, injected intravenously with 1.5 grams of thiosanimine, following which the leucocyte count rose in twenty-four hours from 6500 to 21,000 per cubic millimetre. One part of vaccine emulsion in water was now mixed with an equal quantity of serum obtained by bleeding this goat. The bacterial counts were as follows: Immediately after the mixture, 9736; twenty-four hours later, 4564; then a progressive increase. When, however, to this combination an equal amount of normal serum was added, rapid diminution in the bacterial count took place. Thus, immediately after mixing the count was 9700; two and one half hours later it was 2520; after twenty-four hours it was 873, and in one hundred and forty-four hours the plates were sterile. From this experiment it is safe to conclude that when a sufficiency of normal serum is used all the bacteria in vaccine emulsions can be destroyed; but there seems no reason to assume that serum from blood containing many leucocytes is more effective than normal serum. On the other hand, it would appear that normal serum is capable of destroying various bacteria, including the pyogenic cocci, in the presence of the constituents of the vaccine emulsion.

This result was extended by testing the exudate from a human

lung in condition of red hepatization against normal serum. The exudate, owing to the fact that the lung tissue was not perfectly fresh, contained besides pneumococci and staphylococci certain bacilli, possibly of putrefactive origin. As was to have been expected from the results of previous experiments, the serum had no marked influence on the bacterial count. And yet, as has been shown, when normal serum is mixed with vaccine emulsion, the pyogenic bacteria contained in the latter are markedly reduced in numbers. I was therefore led by these results to test the combined action of glycerin and serum upon vaccine emulsion.

I have already pointed to the difficulty with which glycerin alone destroys certain pathogenic bacteria, and I have shown that while normal serum can effect this end, the process is in its nature a slow one. Now I have found that when normal serum and glycerin are permitted to act together they bring about the reduction more quickly than either fluid alone; and I have also noted the peculiar result that immune sera are less effective in this respect than normal serum, or, at least, with the latter the reduction proceeds with considerably greater celerity.

This last series of results can be more or less altered by modifying the conditions of the experiment. For example: A watery suspension of vaccine tissue when mixed with normal serum and glycerin will lose bacteria more quickly than with the serum alone. But if an immune serum and glycerin are first added to the emulsion and a mixture of normal serum and glycerin be subsequently introduced, then the loss takes place still more quickly and completely. If, again, a sterile exudate be developed by means of aleuronat and emulsified in an immune serum and glycerin before being added to the vaccine emulsion, and if, a little later, a mixture of normal serum and glycerin be introduced, the vaccine emulsion will fail to give any growth at the end of forty-eight hours.

I studied a pneumonic exudate containing innumerable bacteria in every loop. This exudate, when mixed with glycerin, suffered progressive diminution of bacteria, but all growth did not cease until after thirty-two hours. The addition of normal serum, on

the other hand, to the mixture of glycerin and exudate brought about sterility in the short space of five hours.

It was found that the bactericidal action of various immune and normal sera was increased by the addition of glycerin, and the most favorable mixtures were those containing twenty-five per cent. of this substance. And it was also found that in order to destroy bacteria by means of mixtures of serum and glycerin, the most striking results were obtained when the glycerin was mixed with the immune or normal serum before being added to the material containing the bacteria.

CYTOTOXIC SERUM PRODUCED BY THE INJECTION OF NUCLEOPROTEIDS.

By S. P. BEEBE, New York.

(Contributions from the Huntington Fund for Cancer Research, Department of Experimental Pathology, Cornell University Medical College, New York.)

The phenomena of the production of antisera by the introduction of foreign proteids into the circulating medium of an animal are now so well known as to need no introduction. Such antisera have made possible the biological differentiation of proteids, and have furthermore indicated that the proteids obtained from different organs of the same animal species have individualities which, possibly, may be associated with the functions of those organs. The cytotoxic sera were among the first to be produced, and, as early as 1899, von Dungern[1] suggested the possibility of using an epitheliolysin in the destruction of unrecognizable cancer cells that may remain in the tissues after excision by the surgeon. Since that time a considerable amount of work has been done, and from the claims made in the literature one is led to believe that cytotoxins have been produced for nearly all the various tissues of the body.

In nearly all of this work crushed tissues mixed with blood have been injected into some animal at intervals for varying lengths of time and the antiserum thus produced has been used to produce cytotoxic effect upon some member of the original animal species. A serum made in this way is not specific—that is, it causes changes in other organs than the one from which it was made. It invariably shows hæmolytic and hæmagglutinative properties, which is what should be expected when one considers that blood has been used in making the antiserum. The work which Pearce [2] has done along this line indicates that much of the effect produced by such antisera may be explained by their hæmolytic and hæmagglutinative effects. In order to

obtain a serum more nearly specific, Pearce has washed out the organs by perfusing through the blood-vessels with salt solution, but he finds invariably that antisera produced by such washed organs have strong hæmolytic and hæmagglutinative properties *in vitro;* however, they do not always show the same behavior *in vivo.* Nevertheless, he finds that sera prepared in this way from pancreas and adrenal may cause degenerative changes in the liver and kidney. He says: "Some of these cytotoxic sera have no effect upon organs for which they are supposed to have a morphological affinity, but exert a powerful lytic action on other cells," and he concludes that with the exception of the nephrotoxin "the various cytotoxins studied have no specific action in the morphological sense." Pearce is fully justified in drawing these conclusions from the results of his experiments, and his work must be regarded as the most complete and thorough which has yet been done on the specificity of the cytotoxins. It is evident that to add to the subject as he left it requires some new method of attack.

The cytotoxic sera which Pearce made have been produced by the injection of washed organs, and the antibodies thus formed have been developed for the proteid constituents of the cells used for immunization. Without doubt, even in the washed organs there are proteids which closely resemble those found in the blood, together with others that have been built up into complexes characteristic of the particular group of cells used. There must be a physico-chemical basis in the liver, pancreas, and kidney cells which enables them to perform their varying functions. Some portion of the proteid constituents of the tissue must be characteristic for that organ. When we consider that the precipitin or biological test will distinguish between two proteids as closely related as serum globulin from the cat and from the dog, it seems reasonable to believe that two organs such as the kidney and pancreas must contain proteids that are sufficiently characteristic for those organs to be distinguished by one of the biological methods now in use for differentiation. The writer has undertaken the present work in the hope of being able to develop from certain of the proteid constituents of

various organs, cytotoxic sera having a higher degree of specificity than can be obtained with the full cells.

The accepted biological principle at the present time is that the nucleus is the most important morphological and physiological portion of the cell; and hence it follows that its chemical constituents must be those most necessary for nutrition. In accordance with this idea the writer has isolated nucleoproteids from various tissues and has produced antisera by their injection. The present paper deals with the methods of producing, and some of the results obtained with, such antisera.

On studying the literature one finds that few attempts have been made to obtain antibodies to nucleoproteids, and these attempts have been in nearly every case negative. Ide [3] in discussing this point in 1902 said that from such negative results "one fears that it is very difficult to form antibodies against the most important substances of bacilli." His pupil Malengreau found it impossible to demonstrate an antibody after giving a rabbit nine large doses of thymus nucleohiston. He quotes Pick as being unable to show an antibody after giving ten doses of nuclein from the colon bacillus. More recently Galeotti [4] has claimed to obtain an antiserum having considerable immunizing power for the anthrax bacillus by the injection of its nucleoproteids. Levene [5] has made some experiments on the production of hæmolytic sera from different constituents of erythrocytes which favor the belief that nucleoproteids may be effective in forming antibodies. The following substances were used by him: crystallized hæmoglobin, aqueous extract of the red cells, residue from the alcohol-ether extraction, neutral sodium chloride extract, sodium bicarbonate extract. The results were all negative except for the bicarbonate extract, which gave a serum having a strong hæmolytic power. The filtrates from the partial digestion of erythrocytes by pepsin-hydrochloric acid and by a sodium carbonate-trypsin mixture were used. The latter filtrate gave a serum having approximately the same hæmolytic effect as the sodium carbonate extract; the filtrate from the pepsin digestion gave a very much weaker serum. From the well-known solubilities and behavior of the nucleoproteids on digestion one

can readily see that those extracts which gave the active serum must have contained compounds of nucleic acid.

During the progress of the study which forms the basis for this paper, Bierry and Mayer [6] reported the production of cytotoxic sera by the injection of nucleoproteids from the liver and kidney. This work has, it appears, been reported at the meetings of various societies in France, but a complete report has, to the writer's knowledge, not appeared, so that it is difficult to say how closely it resembles his own. Comments will be made on the work of Bierry and Mayer at appropriate places in this paper.

The nucleoproteids are, as is well known, compounds of nucleic acid with proteid; and when the name was first applied it was supposed that they were the proteids of the cell nuclei. It is by no means certain, however, that the usual method of preparation gives as a product those proteid-complexes which are characteristic of the cell nuclei. There is no doubt that the nucleic acid portion of the compound exists chiefly in the nucleus, but precise proof is lacking as to whether the proteid part of the compound comes from the nucleus or from the general cell protoplasm. The problem is not a simple one. Kossel [7] believes that what we recognize as chromatin is nucleic acid combined with more or less proteid, together with some free nucleic acid. The smaller the proteid content of the combination, the more closely does it resemble nucleic acid; and we have reason to believe that the proteid content in the chromatin of the same cell nucleus may be a variable one. We know that nucleic acids can combine with varying quantities of proteids, and the character of these compounds varies with the amount of proteid thus combined. Pepsin digestion breaks off a considerable amount of proteid from the nucleoproteids, leaving the so-called nucleins, which are bodies richer in phosphorus and resistant to pepsin digestion, but still containing some proteid. Osborne [8] believes that the combination of nucleic acid with proteids forms a salt, the character of which is given by the proteid, and that the conditions governing this combination are not radically different from those determining the combination of inorganic acids and

bases. Quite a variety of such salts or protein nucleates differing in the amount of proteid which they contain can be obtained from the same tissue. The nucleic acid probably exists in the cell combined with proteid, but we simply have no proof that the compound which we isolate by the ordinary methods is identical with the one preformed in the cell. From the extensive studies which have been made of the chemistry of the nucleic acids, we know that those obtained from the kidney, liver, spleen, and pancreas show considerable variations, so that, chemically at least, the nucleoproteid which we isolate from any given organ is not precisely identical with that from any other organ. We may reasonably expect, therefore, to obtain sera having a higher degree of specificity by inoculating into some animal of an alien species nucleoproteids derived from different organs.

Preparation of the Nucleoproteids.—The usual method was followed in isolating the nucleoproteids used for preparing the antisera described in these experiments. The organs were sliced thin and placed in jars of running water to remove as much blood as possible. Only the parenchyma of the organs was used, omitting the large blood-vessels of the liver and kidney, and after thorough washing the slices were ground to a fine pulp in a hashing machine, two volumes of water added, and the mass extracted in a cold room for twenty-four hours, chloroform being employed to prevent bacterial developments. In the later work physiological salt solution was substituted for water. After straining through cloth, the fine tissue fragments in the filtrate were removed by centrifugation. The powerful centrifuge used for this purpose gave a perfectly clear extract from which the nucleoproteids were precipitated by the addition of acetic acid up to a frank acid reaction. The precipitate was allowed to settle in large hydrometer jars in a cold room for twenty-four hours, when a large part of the supernatant fluid could be siphoned off. The precipitate was now centrifugated and repeatedly washed with physiological salt solution until the wash-water was perfectly clear. The washing was very readily accomplished by using the centrifuge, for which purpose it was found best not to run the machine too long so as to pack the precipitate too hard

in the tubes. After thorough washing, the precipitate was suspended in normal salt solution and a little sodium carbonate added to dissolve it. Acetic acid was next added to acid reaction and the resulting precipitate again thoroughly washed. The process of dissolving, re-precipitating, and washing was again repeated, the final precipitate being the substance used for injection into animals. In some cases this final precipitate was dissolved before inoculation in 0.5 per cent. sodium bicarbonate solution and kept in the refrigerator. A little chloroform being added, it was noticed invariably that a fine deposit settled out after some weeks, a change probably due, in part, to the well-known coagulating action of chloroform. The temperature of the refrigerator was always very near 0° C. and the tubes were occasionally congealed. In no case has such a solution been kept for more than six weeks. A considerable part of the proteid prepared for the work has been dried at 40° C. in the apparatus already described elsewhere. This dried proteid has been found to dissolve readily in a 0.5 per cent. sodium bicarbonate solution, and the serum made from it is precisely similar in its action to that made from the proteid kept in solution.

Those who have worked with nucleoproteids will appreciate the difficulty of washing and filtering these substances; and hence it is the writer's belief that the powerful centrifuge [9] used in his experiments has enabled him to get a pure product with less change due to autolysis than has been the case with previous writers. The whole process as here given can be completed in thirty hours from the time the fresh organs are received, during the larger part of which time the tissues are in the refrigerator.

Properties of the Nucleoproteids.—The fresh products prepared in the manner described were found to exhibit the property, characteristic for nucleoproteids, of causing intravascular clotting of the blood when injected intravenously. Determinations of phosphorus were made on two samples of each proteid prepared at different times. The average results are given in the following table:

Nucleoproteid from	liver,	2.3	per cent. phosphorus.
" "	kidney,	5.2	" " "
" "	pancreas,	5.4	" " "

From these figures it is evident that if one follows the scheme of Kossel and Lilienfeld,[10] the final product might more properly be called a nuclein. The phosphorus percentages are much higher than are usually found for nucleoproteids. The method of preparation has evidently been such as to split off a large part of the proteid from its combination with nucleic acid. The amount of proteid which combines with the acid when the two are precipitated from a common solution varies with the concentration of the solution. The first precipitate caused by the addition of acetic acid does not completely dissolve on making the suspension alkaline, and in the succeeding operations of precipitation and washing, proteid is lost and the percentage of phosphorus is increased. This behavior has been well described by Bang.[11] The final product is, therefore, in its phosphorus content more nearly comparable with the so-called nucleins than with the nucleoproteids, though it would seem that the essential difference between the two substances consists in the quantity of proteid combined with the nucleic acid. The fact that these bodies are so rich in nucleic acid leads one to believe that the production of antibodies may perhaps be caused as much by the acid portion of the compound as by the proteid.

The color reactions which these substances gave indicate also that only a small amount of proteid has remained in combination with the nucleic acid. The Millon, xanthoproteic, and biuret tests gave only feeble reactions. The Adamkiewicz test gave a good reaction; the Molisch, a very strong reaction.

Preparation of the Serum.—Rabbits only were used in making the serum, and because of the intravascular clotting caused by intravenous injection of nucleoproteids the solutions were always injected into the peritoneal cavity. White rabbits were chosen for the first and gray ones for the later experiments, but no difference could be detected in their reaction to the injections.* The symptoms caused by the injections of nucleoproteids into the white and gray rabbits differed in no respect as far as could

*This difference in the clotting of the blood of white and gray rabbits has never been explained. Halliburton[12] has found that artificial colloids when introduced rapidly cause intravascular clotting in gray rabbits, and, like the nucleoproteids, are without effect on albino rabbits.

be observed. Five injections were made at intervals of six days with gradually increasing quantities of proteid, the final injection being in many cases 0.2 gram, a quantity sufficient to kill several animals if introduced directly into the circulation. Eight days after the last injection the animals were bled. It was found very much better to exsanguinate the animal from the carotid at once than to bleed successively from the ear, because the animals do not stand repeated bleeding and injection well, and furnish a poor grade of serum under the latter circumstances.

The first serum tested was made by the injection of calves' thymus nucleo-histon; and this product proved to be a particularly good one. The precipitin reaction, which was used as a means of determining the presence of an antibody, was positive, a fact which stands in contradiction to the findings reported by Ide. I was, however, able, by using the antiserum in fairly strong concentration, say 1 to 2 or 1 to 5, upon a solution of the nucleohiston, to obtain a definite reaction. The nucleohiston used for this purpose was free from the ordinary nucleoproteid of the thymus gland. An interesting result was obtained with this antiserum by testing it with nucleoproteid from the calves' thymus, for the latter proteid gave in the same concentrations a very much better precipitin reaction than did nucleohiston. This behavior should not be considered surprising. We know that nucleohiston has quite different solubilities * from nucleoproteid; we are also not justified in deciding on the basis of the precipitin reaction alone whether an antibody has been formed. The common statements that albumoses and peptones form no antibodies are based upon precipitin experiments; and it seems not unreasonable to offer the conjecture that if the inoculations were made with peptone, the mother proteid of the peptone might yield evidence of the presence of an antibody in giving this reaction. The formation of hæmolytic and agglutinative sera by the injection of bile and urine points in this direction, for the bile contains cleavage products only of the corpuscles and yet it

* In trying precipitin reactions with nucleohiston the precaution must be taken to make all the dilutions of serum with 2 per cent. sodium chloride solution, for it is precipitated by physiological salt solution.

produces an agglutinative serum which is scarcely weaker than that yielded by the blood itself.

Upon the basis of the precipitin reaction as the test, the statement can be made that definite antisera have been produced for the nucleoproteids of the liver, kidney, and pancreas of the dog. With the stronger concentrations of antiserum, that is, 1 to 2 and 1 to 5, the reaction shows clearly in one hour, while the more dilute solution requires frequently that the tubes be left in the incubator overnight. The difference in the reaction between immune and normal serum was perfectly sharp and definite. The antiserum made in this way did not give a precipitin reaction with the blood-serum of the animal which served as a source of the nucleoproteids. That is to say, the antiserum made from kidney nucleoproteid of the dog did not give a precipitin reaction with normal dog serum. It may be remarked that more striking results were obtained by using freshly prepared proteid for the precipitin reactions; but for making the antiserum, as already stated, no difference was observed whether the dried or the fresh proteid was used. In one case the proteid used for making the inoculations was dried in an oven, which unintentionally reached a temperature of 53° C. for some hours. The product, which was ground very fine in a mortar, did not dissolve in 0.5 per cent. sodium bicarbonate solution and upon injection did not yield an active serum. In the other cases a new oven was used, the temperature of which did not reach above 40°, and no difficulty was experienced in dissolving the proteid.[13]

The writer has attempted to demonstrate cytolytic action of these sera following the method adopted by Flexner and Noguchi[14] in their study of snake venom, but without results. Definite agglutinating action of the serum on suspensions of finely pulped gland was, however, observed. The suspension used in testing this agglutinating action was made by grinding fresh gland finely in a porcelain mortar, mixing with physiological salt solution, and straining through gauze. When the filtrate so obtained is centrifugalized it is found that the sediment is separated into layers, the finer portions being at the top. This finer portion is readily poured off from the coarser fragments below, and

when thoroughly shaken with salt solution the suspension obtained settles very slowly if left to itself or when normal serum is added; immune serum, however, causes it to flock together and settle out much more rapidly. This agglutinating action is, except in strong concentrations, 1 to 1, or 1 to 2, specific. In dilutions of 1 to 4 the flocking can be distinctly seen in one minute.

The question as to whether the serum prepared in the manner described has hæmagglutinative and hæmolytic properties is one of considerable importance in deciding the manner of its action, particularly in view of the statements already made by Pearce. All the antisera thus far made are markedly hæmagglutinative and hæmolytic in vitro in dilutions up to one to five, but in dilutions of one to twenty or above, there is no difference to be detected between the immune and the normal serum. In no case has hæmoglobinuria followed the administration of a serum to an animal. In such quantities as would be used as a therapeutic dose, no blood effects in vitro different from those produced by normal serum were to be seen. The writer is, therefore, unwilling to believe that the toxic effect of this serum, when employed in the living body in such concentrations, as were used in these experiments, depends upon the hæmolytic and hæmagglutinative properties which it shows *in vitro*.

The most interesting results obtained with the immune sera were caused by toxic action upon the living animal. The toxicity being determined, the next step was to ascertain whether the serum is specific and could produce lesions in one organ without affecting injuriously other organs of the body. Various cytotoxins were studied in order to determine this question; but in this communication the results obtained by the injection of liver, kidney, and pancreas nucleoproteid cytotoxins only will be considered. The animals chosen for the inoculations were small adult fox-terriers, which had been kept under observation for some time previous to inoculation; the urine, moreover, was tested to make sure that no functional derangement existed.

The serum was given in some cases intraperitoneally in small and repeated doses, and in others intravenously in one large dose.

The small doses consisted of injections of from one and one half to two cubic centimeters in dogs weighing from five to seven kilos; the large doses consisted uniformly of two cubic centimeters per kilo. The injection of the serum, whether given one way or the other, is followed by a complex of symptoms which may be called a reaction. The animal vomits, and often defecates and urinates; the temperature may rise one degree in the course of the first hour, and there is marked chill followed by a period of sleep. Similar effects, though of slight degree, are produced by normal serum. They cannot be ascribed, I think, to immediate injury of a specific organ, but probably are nervous in origin. No peritoneal lesion was found in any case.

Effects on Kidney, Liver, and Pancreas.—The nephrotoxin made from nucleoproteid causes an acute degeneration of the kidney epithelium, but I have thus far failed to find that it causes those degenerative changes in other organs that have been noted by previous writers as a result of the injection of nephrotoxins. There is, however, almost complete agreement among recent investigators of the subject that of all the cytotoxins nephrotoxin comes nearest to exerting specific action. Pearce[15] says that when prepared from washed kidneys the serum is specific for the kidney and does not affect other organs, "with the exception of the liver, which in some animals exhibited extensive granular degeneration." Woltmann,[16] who evidently made his nephrotoxin from washed kidney, says: "The nephrotoxin perhaps comes nearest to being specific since macroscopically and microscopically marked congestion of the medullary portion of the kidney and pronounced cloudy swelling of the cortex were observed. Similar changes of the same severity were found in no other serum, and it appears that the renal toxin had a specific, perhaps merely irritating, action on the kidney itself."

The following experiment illustrates the action of my serum. A fox-terrier bitch of six kilos body-weight was kept in a cage for three days for observation. The animal was in a perfectly healthy condition as far as could be determined, and examination of the urine showed the kidneys to be sound. On April 8, twelve cubic centimeters of nephrotoxic serum were injected

into the femoral vein, using morphine and cocaine as anæsthetics. The animal showed the usual behavior toward the serum. The urine was collected daily, but no albumin appeared until April 12, when a trace was found by using the acetic acid and potassium ferrocyanide test. The albumin increased in quantity daily, the animal remaining normal in behavior until April 14. On April 15 she ate very little and later in the day vomited; the following day she refused to eat. The urine on this day solidified in the tube when heated and analyses showed that 53 per cent. of the total nitrogen excreted in the urine was in the form of albumin. Abundant granular and hyaline casts also were found. The animal was very sick, the rectal temperature being 98° F. in contrast to 102° previous to the inoculation. On April 17, the rectal temperature had fallen to 95°, and since it was evident that the animal would die shortly, chloroform was administered. The autopsy showed the liver to be slightly congested, but otherwise normal; spleen, normal; kidneys, swollen and pale yellow with punctate hæmorrhages and obscured markings. About fifty cubic centimeters of clear, straw-colored fluid were in the peritoneal cavity.

The *histological* examination * of the tissues showed the following conditions: The *liver* shows a few vacuoles uniformly distributed in the cells and slight congestion of blood-vessels. No marked degeneration and no necrosis occur. The impression given by the section is that the organ is in a normal condition with no pathological change. The *pancreas*, *spleen*, and *lymph nodes* are normal.

The *kidneys* are very much congested, and on microscopical examination the blood appears to be agglutinated, though this appearance may be, and probably is, an artifact. Much cast matter exists in the tubules of the cortex and medulla, and many tubules are filled with blood. The tubules in places are considerably dilated, especially where there are casts. The tubular cells are separated by spaces, somewhat shrunken, often split

* The writer is very much indebted to Dr. Martha Tracy for mounting the tissues for histological examination, and to Dr. James Ewing for the description of the histology cited in this paper.

lengthwise, and eroded. The nuclei stain poorly and some mitotic figures are seen. The glomeruli are normal. Five additional dogs to which this serum was given showed practically the same lesions.

In some cases from 350 to 500 cubic centimeters of bloody serum were found in the peritoneal cavity at the autopsy. If the animal was killed before the process had reached such extreme conditions the peritoneal cavity was free from fluid. There seems to be no doubt from these findings that this nephrotoxin sets up an acute degeneration of the kidney tissue, from which certain secondary changes in the liver might possibly be expected to result, but there was no evidence in these experiments that any of the lesions were caused by hæmolytic or hæmagglutinative properties in the serum.

Regarding hepatic toxin, Pearce [17] states that after its injection "the only change observed was an increased granulation of the liver cells with occasionally slight fatty transformation," while its chief action was to produce more or less extensive fatty metamorphosis of the kidney. Similar conclusions on the nonspecificity of hepatotoxin were reached by Woltmann.

With the serum made by injecting liver nucleoproteids I have produced very serious lesions of the liver, apparently without causing injury to other organs. I shall give an illustrative case: a fox-terrier bitch of 3920 grams body-weight was kept under observation for six days previous to inoculation. The urine was normal in every respect. On May 2 intraperitoneal inoculations of hepatotoxin were begun. These were of two cubic centimeters each, and were made on the following dates: May 2, 3, 5, 10, 12, 13. The animal weighed 4125 grams on May 15 and for some days had been quite sick, but its condition was not so serious as that of other animals undergoing similar treatment, and was far less serious than the condition of the animal described in which the nephrotoxin had been injected. There was no indication at this time of severe hepatic lesions. At no time had the urine showed the least trace of albumin or sugar. The animal was killed by chloroform on May 15. The autopsy made one half hour after death showed the kidneys, spleen, and

pancreas to be normal. There was no fluid in the peritoneum, and all the organs appeared healthy except the liver, which was curiously mottled and showed an evident fatty condition. The following is a brief report of the histological examination of the tissues made by Dr. Ewing:

The *liver*, which is altered pathologically, shows areas one fourth to one half the size of the lobules in which the liver cells are invisible or missing, or the tissue is substituted by a mixture of necrotic liver cells, leucocytes, red blood cells, and detritus. Many of these areas appear to surround central veins. The liver cells in general show intense granular and fatty degeneration, and congestion, most marked in and about the necrotic foci where the red cells appear to be more or less fused. The fusion of red corpuscles may be a post-mortem change. About the hepatic veins round-cell infiltration exists.

The *kidney* shows nothing worthy of note except an accumulation of large round cells about the glomeruli. The tubules, normal in size and not dilated, contain a slight granular coagulum.

The *spleen* is normal.

It is evident, therefore, that this serum has caused a profound change in the liver, but has not damaged other organs. I do not think it probable that the specific action on the liver is to be attributed to the hæmolytic and hæmagglutinative effects of the serum. The difference in the action of this serum and that of Pearce and of Woltmann is undoubtedly to be explained by the fact that in its preparation it has been possible to eliminate from the tissue injected a large amount of extraneous material, as, for example, bile, which previously was included and which undoubtedly influenced the qualities of the serum.

The next experiment was made with a nucleoproteid antipancreatic serum. Pearce [18] has found that an antiserum from the pancreas "did not produce hæmoglobinuria but a persistent albuminuria with fatty degeneration of the kidney. The strongest dose given caused also well-marked focal necroses of the liver with evidence of red-blood-cell thrombosis. No change in the pancreas cells could be demonstrated."

The following example shows the action of the serum made

from pancreas nucleoproteids: A white fox-terrier bitch was kept under observation for some days to be sure that she was in healthy condition. On May 25, using morphine and cocaine as anæsthetics, 15 cubic centimeters of the pancreas serum were injected into the femoral vein. The animal soon recovered and lived in a perfectly healthy condition until June 3, when she refused food. On the next day, June 4, she had marked indigestion with diarrhœa, which steadily grew worse, and on June 5 she was so badly off that she was chloroformed. At no time had there been any albumin in the urine, but beginning on May 30 the urine gave a partial reduction of Fehling's solution, which condition continued till the end of the experiment. By a partial reduction I mean the formation of an abundant green precipitate, but not the typical red deposit of cuprous oxide. The autopsy showed the internal organs to be in a normal condition except the pancreas, which was softer and redder than usual. The stomach and intestine contained a small amount of black, slimy material similar to that vomited and defecated during the preceding 48 hours. The urine taken from the bladder at the autopsy contained no albumin, but gave the usual green precipitate with Fehling's solution. The histological examination of the organs by Dr. Ewing showed the liver, kidney, and spleen to have been quite normal. Of the pancreas it must, I regret, be recorded that the fixation of the tissues was imperfect, thus making the study unsatisfactory. No explanation can at present be offered for this failure.

The findings in this case are interesting, in spite of their inconclusiveness, in showing that a serum made with pancreatic nucleoproteid may act injuriously upon the pancreas without, at the same time, disturbing other organs. Another dog to which the pancreatic serum was given showed similar symptoms, and there was a similar difficulty in obtaining satisfactory sections of tissue for examination. In all of this work the Müller-formol fixing fluid has been used.

A point of considerable interest is seen in the latent period between the administration of the serum and the first appearance of the symptoms indicating action on some particular organ.

This period varies from four to ten days, depending upon the manner of giving the serum and to some extent upon the animal. When the nephrotoxin has been given intravenously albumin appears in the urine in most cases on the fourth day, but at that time it appears as a mere trace only and requires a delicate method to bring it out. The quantity increases rapidly until the eighth or the ninth day, by which time the kidneys have been so badly injured as to place the animal beyond recovery. If the serum is given in small daily doses intraperitoneally, the effects are delayed for some days, apparently until there has been a sufficient accumulation of the toxin. When injected into the peritoneum, the quantity of serum needed to produce toxic effects is greater than with intravenous injection, and should the treatment be stopped after two or three doses have been given intraperitoneally the animal may show no lesions at all, which would indicate that the organism possesses some degree of resistance to the toxin. What has been said of the nephrotoxin holds equally true for the liver and pancreas toxins.

The results which are given in this paper are based upon five experiments in which nephrotoxin, three in which hepatotoxin, and two in which pancreas antiserum were administered. They provide, it seems to me, strong indications that cytolytic sera having a high degree of specificity can be prepared from the nucleoproteids of tissues. Such a thing as absolute specificity under all conditions has never been demonstrated and probably never will be, but it does, nevertheless, seem possible, in the light of these results, to make a serum which will act primarily on a given organ. The demand that a serum, in order to be called specific, shall limit its injury to one organ only is unreasonable on the basis of the well known altruistic relations of the viscera.

Bierry and Mayer[19] note the following in regard to the specificity of the serum which they made from nucleoproteids of the kidney: "L'examen histologique fait par M. Aug. Pettit a montré que les reins presentent des lesions considérables et systematisées affectant les glomérules et les cellules des tubes sécréteurs, mais en même temps d'autre organs (foie, centres

nerveux) sont atteints. L'effet du serum nephrotoxique n'est donc pas rigoureusement spécifique." These statements were not seen by the writer until after much of his work had been done. The results that have been obtained thus far with the nephrotoxin have not confirmed the work of Bierry and Mayer in this respect. The liver has in some cases been slightly congested as in the case cited, but no degenerations occurred. It has been the experience of nearly all investigators that the nephrotoxin is more specific than the hepatotoxin, but Bierry and Mayer have found the reverse to be true. In the following words[20] they describe the lesions produced by their hepatotoxin made from nucleoproteids of the liver: "L'action des injections se traduit par l'apparition de lésions histologiques dont l'examen a été publié ici même par M. Auguste Pettit et l'un de nous. Ces lésions consistent en dégénérescences graisseuse, vacuolaire et granuleuse du cytoplasma des cellules hépatiques. Les autres organes (rein, pancréas) ne sont pas lésés." In the absence of details it is impossible to do more than quote their statements without trying to effect a reconciliation.

In much of the work on cytotoxins the chemical side of the subject has been lost in the maze of pathological considerations involved. The cell has been treated as though it was a definite chemical molecule; but it is my opinion that the time has now arrived when we can well afford to cease dealing with such complex factors as the intact cell and try to find out the details of this interesting matter. The work is being continued in the hope of answering some of the many questions which it has suggested, and I hope to contribute further facts regarding the manner of the action of these sera, together with some practical applications which they may have.

It is a pleasure to record my appreciation of the kindness of Dr. Geo. A. Wallace and Dr. R. A. Hatcher, who furnished me with most of the organs used in this work.

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A CONTRIBUTION TO THE TECHNIQUE OF MAKING THE ECK FISTULA.

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The work of a number of investigators upon the nitrogen metabolism of dogs has relied quite extensively on the establishment of a fistula known as Eck fistula between the vena portæ and the vena cava inferior. The ingenious method recently described by J. E. Sweet[1] is a very material improvement over previous ones in use.

We have thought it of value at this time to place on record our experience in making the Eck fistula, especially since, after several trials with the older methods and with modifications of them, we also came to devise a special technique which would seem to have certain advantages.

In view of the recent publication by Sweet, in which a complete bibliography is given, it does not seem advisable to discuss the older methods here. It is sufficient to state that the performance of them, as well as of a snare operation which we devised earlier, is beset with many uncertainties and complications.

As chief among these, hæmorrhage may be mentioned, and this results from the difficulty of making the opening at the proper place and of right size, without unnecessary injury to the vessel walls. Another great drawback comes from the handling of scissors equipped with wires within a limited space and in a deep-seated area of operation, and the extreme tediousness of the operation itself.

If the hæmorrhage can be controlled, the operation itself is rendered comparatively simple. The following experiments were directed to the overcoming of this difficulty.

[1] *The Journal of Experimental Medicine*, 1905, vii 163.

In order to determine the effects of clamping the vena portæ and vena cava inferior, the following operations were performed.

No. 15, male, mongrel, 8 kilos weight. The portal vein was clamped by compression with soft-rubber tubing for two periods of ten minutes each, with one five-minute intermission.

At the end of the experiment the pulse was 90, respiration 94, temperature 99.8° F. The abdomen was closed, the dog recovering as from an ordinary abdominal section.

No. 16, female, mongrel, 5 kilos weight. The same procedure was followed as in No. 15, except that both the portal vein and the inferior vena cava were clamped, the latter above the entrance of the renal vein, the former above the entrance of the splenic vein.

The results were the same as in No. 15. The pulse rate increased to 100, the respiration to 90, at one period. Perfect recovery followed closing the abdomen.

It was evident then that clamping the vena portæ and vena cava inferior in this manner was followed by no serious consequences. We therefore proceeded to make the Eck fistula as follows:

An incision is made three inches downward from the juncture of the right costal border and the outer edge of the rectus muscle. The vessels are exposed by drawing the intestines toward the left side. A silk ligature is passed around the vena portæ in the space between the bifurcation and the point of entrance into it of the vena pancreatico-duodenalis. This space is usually short, averaging from five to seven millimetres. It is sometimes difficult to locate the vena pancreatico-duodenalis.

The parietal peritoneum overlying the vena cava inferior is cut through and the vessel separated from its bed for one centimetre at two points: one being just above the point of entrance of the renal vein, the other three to five centimetres upwards toward the liver. Between these points one lumbar vein enters, which is tied. In some cases it is necessary to separate the cava from its bed between these two points in order to draw it near enough to the portal vein.

The vena portæ is separated in like manner from the mesenteric envelopment, from the junction of the splenic vein below to that of the pancreatico-duodenalis above.

For controlling the blood current, two pieces of soft-rubber tubing, from five to eight millimetres in diameter and thirty-two centimetres long are used. Two pieces of glass rod of equal length can be easily slipped into each so that their point of contact is at the mid-point of the tube. One of the glass rods

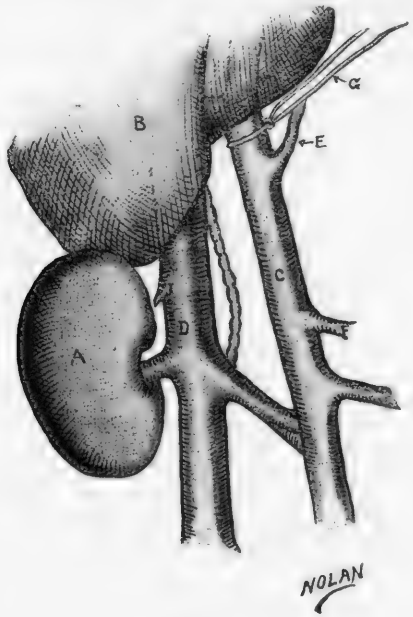

FIG. 1.

A, Kidney. B, Liver. C, Vena Porta. D, Vena Cava. E, Vena Pancreatico-Duodenalis. F, Vena Splenica. G, Lig. about Vena Porta (loose). I, Lumbar Vein.

is slipped into one end of a tube, across the end of which a clamp is placed, while the other end of the tube is carried beneath the vena cava and vena portæ just above the renal and splenic veins. The vessels are brought to lie opposite the middle of the tube. The second glass rod is then slipped in until it touches the first and a clamp is placed over the end of the tube to hold it. By approximating the outer ends of these rods the blood current is controlled and the vessels are held in close

apposition for the suturing. Another tube is placed in like manner just below the entrance of the vena pancreatico-duodenalis. The seat of operation in the veins is between these two clamps.

In order to make the first line of sutures elliptical, a temporary suture is placed midway between the clamps and at a point one-

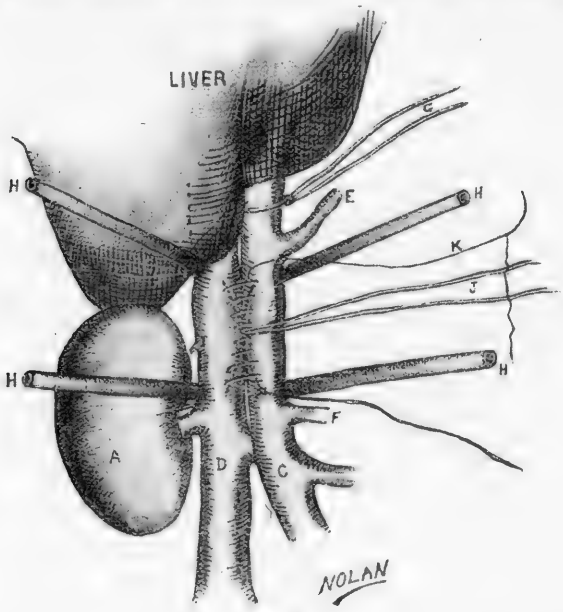

FIG. 2.

A, Kidney. B, Liver. C, Vena Porta. D, Vena Cava Inferior. E, Vena Pancreatico-Duodenalis. F, Vena Splenica. G, Lig. about Vena Porta (loose). H, Rubber Tube Clamps. I, Lumbar Vein. J, 1st. Temporary Suture. K, 1st. Running Suture (loose.)

fourth way around and between the veins, so that when traction on it is made this point will be drawn up. The suture passes through the wall of the portal vein and through the adventitia and media of the inferior cava. By pulling gently upon it and closing the clamps which are held by an assistant, a line of running sutures is inserted from one and a half to three centimetres long. This operation requires five to ten minutes. The

temporary suture is withdrawn and the clamps loosened. Five minutes is allowed for the congestion to be relieved, during which time two other temporary sutures, one at the mid-point on top of each vein, are placed.

The clamps are then closed. The temporary suture in each vessel is separately drawn so as to put the wall on a stretch be-

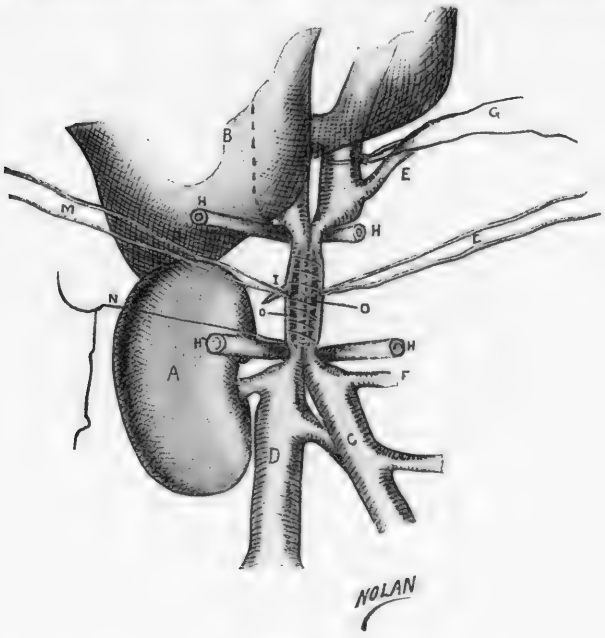

FIG. 3.

A, Kidney. B, Liver. C, Vena Porta. D, Vena Cava Inferior. E, Vena Pancreatico-Duodenalis. F, Vena Splenica. G, Lig. about Vena Porta (loose). H, Rubber Tube Clamps. I, Lumbar Vein. L, M, Temporary Sutures to hold edges of slits in vessels. N, Running Suture (loose). O, Slits in Vessels.

tween this point and the first line of sutures. A fine pair of scissors is then used to make a slit in each vessel from one to two and a half centimetres long. The two outer edges of these slits are sewed together by a running suture, after which the temporary sutures and clamps are removed.

Six dogs were operated on in this way, with the following results:

1. One dog showed a collateral circulation, and in this animal the portal vein had been tied by mistake distally to the point of entrance of the vena pancreatico-duodenalis. The collateral cir-

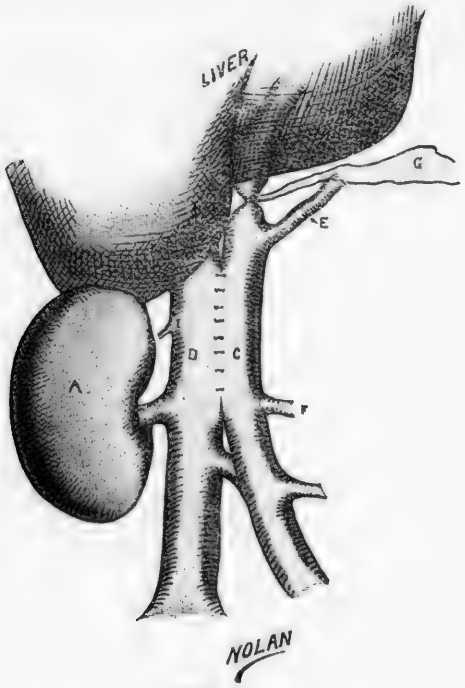

FIG. 4.

A, Kidney. B, Liver. C, Vena Porta. D, Vena Cava. E, Vena Pancreatico-Duodenalis. F, Vena Splenica. G, Lig. about Vena Porta (tied).

culation consisted in an anastomosis between the pancreatico-duodenalis and the splenic vein. This dog suffered a series of interesting convulsions to be described elsewhere.

2. One dog died of a septic pneumonia following a secondary operation on the veins, in which an incision was made through stitch abscesses in the abdominal wall.

3. One dog died twelve hours after operation in coma. This dog recovered well from the anæsthetic, walked about, was catheterized, but gradually sank into coma. Autopsy showed a patulous fistula without thrombosis and with the portal vein ligatured.

4. Two dogs had convulsions following a meat diet. One died during these convulsions, the other became to all appearances normal and was killed for autopsy.

The first of these two showed a fistula one and a half centimetres, the second one centimetre, across. In neither had a collateral circulation developed.

5. The sixth dog is still living at this writing and is in good condition.

In order to prove the non-existence of collateral circulation, these dogs were injected through a canula placed in the inferior vena cava below the fistula, pointing toward the heart. A cut was made in each lobe of the liver and the inferior cava clamped above the liver.

The injected fluid, as was expected, came back and flowed out through the hepatic veins on the cut surfaces. The cava was then clamped between the fistula and the liver, and the injection continued. The fluid was now passing through the fistula into the portal circulation which was completely injected, but none appeared on the cut surfaces of the liver. Hence it was proven conclusively that no communication between the extra-hepatic and intra-hepatic portal circulation existed.

We were unable to demonstrate the collateral circulation described by v. Kaltreu in the ligamentum hepato-duodenale, and can see no reason for its formation provided the fistula be of sufficient size.

By the above described technique the hæmorrhage attendant upon other methods is lessened, the operation is simplified, and the duration is shortened to from three-quarters of an hour to one hour.

The results of the operation are as good as, or better than, by the older methods. In my hands all the dogs survived the operation.

It is, of course, essential that the needles and suture material be of the finest compatible with the necessary strength, and that the operators adhere strictly to the usual precautions for an aseptic technique.

As has been noted, several of the dogs with fistulas exhibited symptoms very like those described by Pawlow, Nencki, and others. A chemical examination of the urine of these dogs was made by Drs. Macleod and Haskins. The results of these observations, and a discussion of the possible relationship of the convulsions to carbamate poisoning, as suggested by Nencki, will be published in the near future.

I am greatly indebted to Dr. J. J. R. Macleod for assistance and suggestions which he kindly extended to me. I wish also to express my thanks to Messrs. Haskins, Lewis, and Schultz, and to Messrs. Wilcox, Miller, and Cummer for various acts of assistance.

THE PRESENCE IN THE BONE-MARROW OF ENZYMES RESEMBLING THOSE OF LEUCOCYTES.

By EUGENE L. OPIE, M.D.

(*From the Rockefeller Institute for Medical Research, New York.*)

The leucocytes of an inflammatory exudate contain an enzyme which even after death of the cell is capable of digesting fibrin, coagulated serum, and other proteid substances. This digestion, as Fr. Müller [1] has shown, occurs more rapidly in a weakly alkaline medium. It is doubtless such enzymes that cause intracellular digestion of proteid material engulfed by phagocytic leucocytes.

In a preceding article [2] I have shown that cells carefully separated from the serum of an inflammatory exudate obtained by injecting aleuronat into the pleural cavity of a dog cause much more active digestion of coagulated serum in a two-tenths per cent. solution of sodium carbonate than in acetic acid diluted to the same strength. The cells of an exudate removed twenty-four hours after the injection of aleuronat are almost wholly polynuclear leucocytes with fine granulation. Since the serum of the exudate and of the blood exerts an anti-enzymotic action which in an alkaline medium inhibits the digestion of proteid, it is necessary to free the cells from serum in order to study the behavior of the ferments which they contain.

To determine the source of the ferments contained in the polynuclear leucocytes, attention has been directed to the bone-marrow. A suspension of cells obtained by finely dividing the bone-marrow of the dog and repeatedly washing by centrifugalization with normal salt solution (0.85 per cent.) has been allowed to act upon serum diluted with salt solution and heated in order to destroy its anti-enzymotic action. The amount of coagulable proteid transformed by digestion into non-coagulable form has

[1] *Zeit. für. klin. Med.*, 1888, xiii, 149.

[2] *Jour. of Exper. Med.*, 1905, vii, 316.

been measured by the Kjeldahl method, the amount of nitrogen in the filtrate after coagulation by heat being determined before and after digestion for several days at 37° C. The details of the method employed have been described in the article previously mentioned. The nitrogen contained in the filtrate is represented by cubic centimeters of $\frac{1}{10}$ N. sulphuric acid. The cells obtained from the bone-marrow have been suspended in nine times their volume of 0.85 per cent. salt solution. In order to supply proteid material upon which ferments may act, serum of the blood or of a sterile exudate obtained by injection of aleuronat into the pleural cavity of a dog has been used. This serum has been diluted with salt solution and heated for a half hour at from 75° to 85° C.

Nitrogen of uncoagulable proteids in 5 c.c. suspension of bone-marrow +10 c.c. heated serum represented by.....	5.8 c.c.
Same with 0.2 % acetic acid at 37° C. 4 days.....	10.6 "
Same with 0.2 % sodium carbonate at 37° C. 4 days.	23.25 "

Hedin and Rowland [3] have shown that the juice expressed from the spleen of the beef, horse, pig, and sheep undergoes much more active autolysis in an acid than in an alkaline medium. The expressed juice has normally an acid reaction; addition of acetic acid (0.25 per cent.) or hydrochloric acid (0.1 per cent.) increases the activity of proteid digestion, while addition of sodium bicarbonate (0.2–0.37 per cent.) diminishes the amount of digestion, which is, however, still considerable. The same observers [4] subsequently studied the autolysis of other organs under the same conditions and found that the lymphatic glands, the kidneys, and the liver contain proteolytic enzymes which act most efficiently in an acid medium. Autolysis of juice expressed from voluntary muscle occurs in relatively slight degree and is not materially favored by either an acid or an alkaline reaction. The heart muscle contains an enzyme which resembles that of other organs and is more active in an acid medium. Hedin [5] has succeeded in separating almost completely two

[3] *Zeit. für phys. Chem.*, 1901, xxxii, 341
[4] *Ibid.*, 531.
[5] *Jour. of Phys.*, 1904, xxx, 155.

enzymes contained in the spleen; to that which acts in an alkaline medium he has given the name lieno-α-protease, while that which acts in an acid medium he has designated lieno-β-protease. He has shown that these ferments not only digest the substance of the cells which contain them but are capable of breaking down other proteids such as fibrin, casein, and coagulated blood serum.

To compare the proteolytic ferments of the bone-marrow with those of other organs, tissues minced by scraping with a knife have been forced through a fine sieve and suspended in normal salt solution. The parenchymatous cells thus obtained have been washed several times by centrifugalization with many times their volume of salt solution to free them from the serum of the blood contained in the tissue. They have been finally suspended in nine times their volume of salt solution. In the following experiment suspensions of cells have been subjected to autolysis under toluol.

	Liver.	Kidney.	Spleen.
Nitrogen of uncoagulable proteids contained in 5 c.c. suspension of cells represented by	1.25 c.c.	1.05 c.c.	1.5 c.c.
Same with 0.2 % acetic acid at 37° C. 5 days..	3.35 "	2.1 "	3.65 "
Same with 0.2 % sodium carbonate at 37° C. 5 days.....	2.25 "	1.9 "	2.8 "

When carefully separated from the serum, cells from the liver, kidney, and spleen undergo more active self-digestion in an acid than in an alkaline medium. To test the ability of these cells to cause digestion of proteid material not contained in their own protoplasm, coagulated serum has been added to the suspensions previously employed. Mixtures containing five cubic centimeters of the suspensions of cells and ten cubic centimeters of diluted and coagulated serum have been digested under toluol five days at 37° C.

	Liver.	Kidney.	Spleen.
5 c.c. cells + 10 c.c. coagulated serum + 0.2 % acetic acid.....	12.35 c.c.	8.15 c.c.	14.15 c.c.
5 c.c. cells + 10 c.c. coagulated serum + 0.2 % sodium carbonate.....	5.9 "	6.4 "	7.3 "

The nitrogen of uncoagulable proteids in 10 c.c. coagulated serum is represented approximately by 5.2 c.c. $\frac{1}{10}$ N. acid.

The results obtained accord with those of Hedin and Rowland, and show that the proteolytic enzymes of the liver, kidney, and spleen digest more actively in an acid than in an alkaline medium. In this respect these enzymes differ from those contained in the bone-marrow.

To compare the activity of the enzymes of the bone-marrow with those of other organs, suspensions of cells prepared by the same method were mixed with diluted and coagulated serum.

	Bone-marrow.	Spleen.
Nitrogen of uncoagulable proteids in 5 c.c. cells + 10 c.c. coagulated serum represented by.....	6.05 c.c.	3.85 c.c.
Same with 0.2 % acetic acid at 37° C. 5 days.....	17.2 "	13.7 "
Same with 0.2 % sodium carbonate at 37° C. 5 days..	21.5 "	9.95 "

The cells used in this experiment were allowed to undergo autolysis without addition of serum.

	Bone-marrow.	Spleen.
With 0.2 % acetic acid at 37° C. 2 days.....	3.45 c.c.	3.05 c.c.
With 0.2 % sodium carbonate at 37° C. 2 days	3.6 "	1.8 "

Almost complete digestion of the available proteid doubtless has been produced by autolysis of cells except when splenic cells have been digested in an alkaline medium. It is evident that the proteolytic enzymes in both spleen and bone-marrow are capable of actively digesting the proteids of heated serum. The enzymes of the bone-marrow are much more active than those of the spleen, and even in an acid medium cause more digestion.

The following experiment in which bone-marrow is compared with lymphatic gland has given similar results.

	Bone-marrow.	Lymphatic Gland.
Nitrogen of uncoagulable proteids in 5 c.c. cells + 10 c.c. coagulated serum represented by.....	4.0 c.c.	3.55 c.c.
Same with 0.2 % acetic acid at 37° C. 5 days.....	14.65 "	7.55 "
Same with 0.2 % sodium carbonate at 37° C. 5 days	23.65 "	4.3 "

The foregoing experiments demonstrate that the bone-marrow

contains proteolytic enzymes which are more active than those of the spleen, lymphatic glands, liver, and kidneys. Unlike the enzymes of these organs, those of the bone-marrow act more efficiently in an alkaline than in an acid medium, and in this respect resemble the proteolytic ferments contained in the leucocytes of an inflammatory exudate. Since the cells of such exudates, especially when obtained within twenty-four hours after the injection of an inflammatory irritant, are almost wholly polynuclear leucocytes with fine granulation, there can be little doubt that the enzymes which they contain are identical with those demonstrable in the bone-marrow. Since morphological studies have demonstrated that leucocytes of this type are formed in the bone-marrow, this organ may be regarded as the source of the enzyme which both contain.

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